cells. We investigated the effect of immunological gene therapy with IL-15 protein using alternative IL-15 cDNA with high translational efficiency.

Method and Results: In a malignant model using BALB/c mice and syngeneic Meth A fibrosarcoma, two expression vectors carrying murine IL-15 gene were constructed for use in tumor immunotherapy, one utilizing IL-15 cDNA with alternative exon 5 and the second utilizing IL-15 cDNA with normal exon 5. The first vector induced the production of large amount of IL-15 protein in Meth A, whereas tumor cells transfected by the second vector produced only marginal level of IL-15 protein. Although in vitro cell growth of both transfectants remained unchaged, inoculation of clones transfected with normal IL-15 cDNA resulted in progressive tumor growth, while clones transfected with alternative IL-15 cDNA led to rejection of the tumor. The clone producing high levels of IL-15 grew progressively in nude mice and anti-CD4 mAb treated mice, while the growth of the transfectants was retarded in anti-CD8 mAb or anti-asialo GM1 Ab-treated mice. Cured mice were shown to have generated immunity against a subsequent challenge with wild type of Meth A but not against Meth 1 tumor cells, another type of fibrosarcoma derived from BALB/c mice.

Conclusion: Tumor therapy based on IL-15 gene transfection was effective against Meth A tumor cells, suggesting a possible application to human neoplasms.

1447 POSTER

In vitro generation of HLA-A2 restricted cytolytic T lympho-cytes using an HLA-A2+ allogeneic SCCHN cell line for lymphocyte stimulation

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Vaccines containing tumor-derived alloantigens able to elicit strong MHC class I-restricted tumor antigen-specific T cell responses in patients with HNC might be as advantageous and easier to prepare than autologous vaccines. To begin to test the hypothesis that antitumor effector T cells can be consistently generated by in vitro sensitization with antigens expressed on HNC cell lines, we established a model system, utilizing HLA-A2+ HNC cell line, PCI-13 pretreated with 1000 IU/ml of IFN-gamma, as a stimulator of allogeneic normal HLA-A2+ T lymphocytes. HLA-A2+ peripheral blood T cells obtained from leukapheresis products of 10 normal donors were sensitized by 4 cycles of co-incubation with irradiated PCI-13 cells in the presence of IL-2, IL-1b, IL-4, IL-6. In 4/10 cases CD8+ T cells lines were generated which were able to lyse PCI-13, and 2 other HLA-A2+ SCCHN targets but not HLA-A2+ non-SCCHN targets, K562 or HLA-A2- tumor targets in 4 h Cr-release assays. Lysis was blocked by anti-CD3, anti-MHC class I and anti-HLA-A2 but not MHC class II Abs. The lines were tested for the frequency of cytolytic T cell precursors (CTL-p) responsive to PCI-13 in limiting dilution assays (LDA) and by ELISPOT. The frequency of PCI-13-specific-CTL-p in the best of four CTL lines was 1.04% in LDA. ELISPOT closely approximated LDA data, with the frequency of T cells able to produce IFN-gamma in response to PCI-13 determined to be 1.4%, and this response was inhibited by anti-MHC class I Abs. The data indicate that CTL-p responsive to class I-presented HNC-associated epitopes in normal donor PBMC and can be expanded in vitro, using cytokines and repeated stimulation with the allogeneic tumor cells. Based on these results, we expect in pending experiments that using HLA-matched allogeneic tumor-derived peptides pulsed onto autologous dendritic cells, it might be possible to generate and reliably quantitate CTL-p in patients with HNC.

1448 POSTER

Modulation of human tumor associated macrophages from malignant effusions with cytokines and proteolytic enzymes

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Tumor associated Macrophages (TAMs) represent a major component of the lymphoreticular infiltrate of human tumors, malignant pleural effusions and malignant ascites. TAMs are functionally involved in anti-tumor defense via cytotoxic activities such as f.i. direct cellular cytotoxicity and release of cytokines. They also have the capacity to affect aspects of the biology of neoplastic tissues like vascularization, growth rate, stroma formation and dissolution. The objective of this study was to investigate the effect of various cytokines (GM-CSF, IFN-g, Il-1b and IFN-a) and a polyenzyme preparation. on the functional activity of TAMs isolated from malignant effusions of patients with ovarian, breast and lung cancer. TAMs were isolated by density centrifugation over a discontinuous Ficoll-Hypaque gradient. Peripheral blood monocyte derived macrophages (PBMMs) – serving as controls – were obtained using a combination of density centrifugation and selective adhesion followed by incubation with GM-CSF. The expression of cytokines was determined on mRNA-level via RT-PCR and on protein level via ELISAs. Biologically active TNF-a as well as cellular cytotoxicity were determined using bioassays. The activation status of TAMs differed markedly from that of PBMMs. TAMs showed a significantly lower Il-1b production and higher TGF-b production. Cellular cytotoxicity was markedly lower in TAMs when compared to PBM derived macrophages. The tested cytokines, especially GM-CSF as well as the polyenzyme-preparation were able to induce and increase the production of TNF-a and to enhance the cellular cytotoxicity. A decreased TGF-b production on mRNA and protein level was observed in TAMs treated with cytokines or the polyenzyme preparation. TAMs are one of the immune system's representatives at the host-tumor interface and reflect in some way the failure of the host to have immunologically controlled the tumor. TAMs represent a promising target to therapeutic intervention. With this study we demonstrated that it is possible to stimulate in vitro the functional activity of TAMs by treatment with cytokines or polyenzyme preparations. This might elucidate of the role of macrophages and especially TAMs in tumor defense.

1449 POSTER

Proteinases reduce metastatic dissemination and increase survival time in $C_{57}Bl_6$ mice with the Lewis lung carcinoma

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Purpose: Although proteases in general are considered to be prometastatic and proinvasive, the aim of presented study is to demonstrate *in vivo* an action of enzymes with different site of action, different substrate specifity and influencing directly cancer cell signalling by an other way, than tissue metalloendopeptidases.

Methods: The effect of combined proteolytic enzymes (trypsin, chymotrypsin and papain), administered by the rectal route, on the metastatic process and the time of survival in $C_{57}Bl_6$ mice with the Lewis lung carcinoma inoculated subcutaneously was investigated.

Results: In the control group, which received no enzyme treatment, 90% of animals died of the metastatic spread of cancer by day 18 after primary tumor extirpation. In Group A, which received the multi-enzyme solution from the time of primary tumor extirpation, 30% of mice died of disseminated cancer by day 25. In Group B, which was treated with the enzymes from 6 days before primary, tumor extirpation, only 10% of animals showed the metastatic process by day 15. In Group C, which received the enzymes from 24 hours after intracutaneous tumor inoculation, no metastatic dissemination was recorded. In these three groups, the enzyme treatment was carried out throughout the experiment. None of the control animals survived till the end of experiment at 100 days. The treated groups A, B and C showed survival till the end of experiment in 60%, 90% and 100% of animals, respectively.

Conclusion: In $C_{57}Bl_6$ mice with the Lewis lung carcinoma transplanted intracutaneously, administration of the enzyme mixture showed anti-metastatic effect. Although only some of the mechanisms of the enzyme effects after administration into the systemic circulation are known, our experiments have shown that these enzymes warrant further experimental studies with the prospect of being used in human medicine in integrated anti-cancer therapy, alongside surgery, actinotherapy and chemotherapy

1450 POSTER

Bioactivity of GM-CSF and IL-2 in cancer patients

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Background: GM-CSF promotes the proliferation and differentiation of professional antigen presenting cells (APC) and may synergistically interact with IL-2 in generating an efficient tumor associate antigen (TAA) specific immune-response. On these bases we designed a pilot study in chemoresistant cancer pts in order to evaluate the toxicity of the treatment with GM-CSF and IL-2 and its effects on biological and immunological parameters. The pts received 150 γ of GM-CSF sc for five days (days 1–5) followed

by sc IL-2 for 10 days (days 6–15) and then by 15 day rest (days 16–30). IL-2 daily dose was escalated, starting from 250 MIU in subsequent groups of three pts, according to the Fibonacci's schedule, every two cycles.

Results: 15 pts (8 colon, 1 lung, 1 pancreas, 3 renal and 1 prostate carcinoma and 1 soft tissue sarcoma) entered the study. Thirty cycles were completed and a maximal IL-2 dose of 2,500 MIU was achieved without significant side-effects. Granulocyte, monocyte, dendritic (CD34+, CD14+, CD80+, CD11c+, HLA-I+, HLA-Dr+) cell, and NK (CD3−, CD56+, CD16+, CD11b+) increase was observed in all pts after treatment. GM-CSF/IL-2 also increased the CD4/CD8 ratio in 13 pts who previously presented an inverted CD4/CD8 ratio.

Conclusions: these preliminary results suggest that GM-CSF and IL-2 combination is not toxic and its biological activity in cancer pts might be useful to support anticancer active TAA-specific immunotherapy by increasing APC activity and T cell immune-response. Supported by a grant from MURST (ex-40%).

1451 PUBLICATION

Long term follow up of 50 patients with metastatic renal cell carcinoma treated with high dose i.v. interleukin. 2

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From 7/89 to 10/93 50 patients (pts) with metastatic renal cell carcinoma (RCC) were treated with rIL-2 at the close of 18×10^6 I.U./m^2/day continuos infusion for 5 days, 2 days rest and 5 additional days every 3 weeks for 2 cycles; in pts with response or stable disease 4 additional 5 days cycles were administered. The pts characteristics were: 35 M, 15 F, median age 59 (32–76) years; median PS 1 (0–2); the metastatic sites were only lung in 20 pts; 20 pts were pretreated. All pts were considered evaluable for toxicity: 38% of the pts had at least 1 G 3–4 episode, 2 treatment related deaths (infection after polmonary toxicity) occurred. A total number of 268 cycles was administred and all pts who could receive at least 1 cycle (45) were considered evaluable fo response. We observed 4 CR, 5 PR, 16 NC, with similar duration (8 months); the median (range) survival (months) of the CR is 86 (7–112), PR 24 (12–76), NC 28 (7–87), PD 8 (1–44); 2 CR pts are still disease free after metastasectomy since they had only one site of relapse (thorax). Out of the prognostic factors considered (sites of disease, total received dose, lymphocitosis and eosinophilia, WHO-PS, age) only the P.S. was correlated with the survival. Lymphocitosis (100% increase of lymphocites count after the first cycle) occurred in 100% of CR, 70% of PR, 100% of NC, 70% of PD and thereafter, in our experience, it couldn't be considered a "biological marker" of response. In conclusion: this long term follow up shows that a small percentage of RCC pts treated with high dose i.v. rIL-2 can have a significative increase of survival and some of them after surgery may be considered cured.

1452 PUBLICATION

Fas ligand (CD95L) induction in human lymphocytes by the apoptosis-inducing mistletoe lectins

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Purpose: Fas ligand (FasL) triggers apoptosis in Fas receptor (Apo-1)-positive target cells. We investigated the expression of FasL, Fas and TNF receptor 1 (TNF-R1) on cultured human lymphocytes and leukemic T and B cells.

Methods: Cell surface molecules were measured by flow cytometry in lymphocytes from 6 healthy individuals, from 4 patients with chronic lymphocytic T or B cell leukaemia, and leukemic Molt-4 cells incubated for 72 h with the apoptosis-inducing mistletoe lectins (ML I and ML III) at 10 ng/ml.

Results: ML significantly induced apoptosis in a fraction of lymphocytes, while in the surviving $CD4^+$ T helper cells, $CD8^+$ cells and $CD19^+$ B cells, FasL and TNF-R1 was upregulated, while the Fas molecule decreased. In contrast, FasL was not induced in leukemic cells. This may reflect distinct 'activation' of the surviving cells, which did not result in a proliferation response as measured by the expression of CD25 and CD71, or nuclear Ki-67 antigens. Surprisingly, the apoptotic cells showed increased level of intracellular IL-4, indicating that apoptosis and tolerance are linked through the production of anti-inflammatory cytokines to prevent deleterious immune responses.

Conclusions: Apart from a direct induction of apoptosis in response to an inhibition of protein synthesis by the enzymic ML A chain, ML treatment may indirectly induce apoptosis in Fas^+ tumour cells through activated $FasL^+$ lymphocytes. As ML-rich whole plant extracts from *Viscum album* L. are applied as an adjuvant in complementary cancer therapy, an implicated clinical relevance of their FasL-inducing properties has to examined carefully.

1453 PUBLICATION

Alteration of expression in c-erbB2, bax, p53, bcl-2, JNK, p21 and PKC/c-myc induces PCD in breast carcinoma after adm. of hexadecyl-PC, antiHER2-mAbs & vinorelbine conjugates

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Purpose: HER/neu gene is overexpressed in 30% of human breast cancers, and it is associated with p53 abnormalities, aneuploidy, intrinsic multidrug resistance due to inhibition of apoptosis, augmented DNA repair mechanisms, DNA synthesis, cell growth, mitotic rate, tumourigenicity and metastatic potentiality leading to poor prognosis.

In this study, we aim to find if there is therapeutic utility of anti HER2-IgG bearing fusogenic immunoliposomes consisting of PKC inhibitor-hexadecylPC with encapsulated anti-mitotic vinorelbine-tartrate against advanced breast carcinoma, which exhibits overexpression of tumour suppressor gene p53, and protooncogene HER-2/neu, due to mutations.

Methods: A patient with axillary node metastasis, secondary to a breast primary has been treated with mastectomy. From this specimen, tissue was treated with collagenase and tumour cells were isolated. Paraffin-embedded formalin fixed tissue was analysed by IHC with relevant antibodies for ER, PgR, HER-2/neu, bax, p21, p53 and bcl-2. JNKmRNA and PKCmRNA were measured by Northern blot. Apoptosis was assayed by transmission electron microscopy. Tumour cells were analysed before and after treatment with vinorelbine encapsulated in antiHER2-IgG bearing fusogenic liposomes consisting of hexadecyl-PC.

Results: The breast carcinoma was identified as hormone independent. After treatment, immunochemical analysis has exhibited upregulation of p21, bax, c-myc and downregulation of c-erbB2, bcl-2 compared to measurements before treatment. Expression of p53 remained enhanced due to mutations in the middle region of exon 6 (AA 212–217) before and after treatment. Post-treatment measurement with Northern blot has exhibited enhanced expression of JNK, and reduced expression of PKC compared to pre-treatment assay measures. TEM has exhibited irreversible D2 stage of apoptotic signs with formation of apoptotic bodies, which are phagocytosed by adjacent tumour cells implying a by-stander effect.

Conclusion: We have achieved to eradicate chemoresistant human breast carcinoma cells by apoptosis mediated by the kinase activity of JNK, circumventing mtp53.

1454 PUBLICATION

Lyophilized whole human melanoma cells enhanced suppressive action of PBMC toward survival of the corresponding malignant cell line in vitro

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Purpose was to determine: a) Does the peripheral blood mononuclear cells (PBMC) six-days-preincubation in nutrient medium with human AB serum with lyophilized human melanoma BG or Fem-x cells affect their antiproliferative action towards the corresponding malignant cell line in vitro and b) Does the PBMC six days preincubation with lyophilized normal PBMC, obtained from healthy volunteer (as a source of allogenous, but not of tumor antigens), affect their suppressive action on the survival of both melanoma BG and Fem-x cell lines in vitro.

Lyophilization of malignant cells, as well as of normal PBMC obtained from healthy volunteer, was done by freezing the suspension of whole cells in nutrient medium with normal human AB serum at −80°C. The frost suspension was dehydrated in high vacuum, in lyophilizer. Determination of the antiproliferative action of the untreated (naive), or of six days stimulated PBMC on malignant cells, was also done by MTT test.

Results showed that six days stimulation of PBMC with lyophilized whole BG cells enhanced their suppressive action towards the survival of BG cells in 17 from 19 investigated cases. Six days stimulation of normal PBMC with lyophilized Fem-x cells enhanced their supression of Fem-x cell survival in 8