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