840 POSTER

Natural killer cell activity against the human prostate cancer cell, PC-3: effects of IL-15, GM-CSF and indomethacin

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Purpose: The direct administration of GM-CSF, and GM-CSF-secreting cancer cell vaccines generated from cancer cells by ex vivo gene transfer, have been shown to promote antitumor immune responses in a variety of animal tumor models, including preclinical models of prostate cancer (PCA), and in human clinical trials. The current study evaluated the effects of the in vitro stimulation of the human prostatic cancer cell line PC-3 with IL-15, GM-CSF and Indomethacin (IM), on the natural killer (NK) cell activity.

Methods: Cytotoxicity assays (51Cr release) were performed at different effector:target (E:T) ratios using IL-2 induced NK cells from peripheral blood against PC-3 target cells previously cultured in the presence or absence of 100U/ml of IL-15, 10ug/ml of GM-CSF or 10-5M/ml of IM for 2 days.

Results: IL-2 induced NK cells displayed an average of 32% of cytotoxicity (E:T ratios of 30:1) against PC-3 target cells. Cytotoxicity increased to values of 73%, 52% and 51% when PC-3 target cells were cultured with IL-15, GM-CSF and IM, respectively.

Conclusions: These results suggest that the immune mediators IL-15, GM-CSF and IM can induce an anti-tumor NK response by cellular modulation of prostate cancer cells, which can be used for the development of new therapeutic strategies against PCA.

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Role of nk activating receptor (NKP30) in immunosuppression of advanced gastric cancer patients

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Purpose: Decreased NK cell activity in advanced gastric cancer (AGC) patients has been observed. NK activating receptors has been reported to play critical roles in anti-tumor effect of NK cells on various cancer cell lines in vitro. To clarify the mechanism of natural cytotoxicity depression, we evaluated the expression status of NK activating receptors of peripheral blood lymphocytes (PBL) from AGC patients in comparison with normal volunteers.

Methods: Twenty-one AGC patients and seven healthy volunteers were included in this study. PBL were separated from heparinized blood by centrifugation over Ficoll-Paque. The cytolytic activity was assessed in a 4-hour 51Cr-release assay in which PBL were tested against the K562 cell line. The expression level of NK activating receptors (NKp46, 30, 44) was measured by semi-quantitative RT-PCR analysis.

Results: NK activity from cancer patients was significantly lower than that of control donors ($p<0.01$). Expression level of NKp46 varied among individuals but that of NKp30 mRNA in AGC patients was significantly lower than control donors. NKp44 mRNA was not expressed in both groups.

Conclusion: NKp30 mRNA expression of PBL in AGC patients was consistently suppressed. These results suggest that NKp30 may play an important role in depressed NK activity of cancer patients.

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Interleukin-2 (IL-2), interferon-A (IFN-A), 5-fluorouracil (5-FU) and vinblastine (VBL) for treatment of metastatic renal cell carcinoma (MRCC)

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Between January 1966 and May 2000, 67 patients with MRCC entered a Phase II study evaluating the efficacy of immunochemotherapy. 62 pts with the following characteristics were evaluated: median age 63, ECOG PS: 1 - 45 and 2 -17 pts. Histology: clear cell carcinoma 46, spindle cell sarcomatoid type 2, mixed type 8, unknown 6 pts. 30 pts had metastases at diagnosis and 22 underwent nephrectomy, 32 pts with localized disease underwent nephrectomy and later developed metastases. Site of disease: lungs 66%, lymph nodes 50%, bone 35%, kidney 13% and liver 8%. Number of sites of metastases/patients: 1 site -39%, 2 sites - 27%, 3 sites -27% and 4+ sites 7%. Treatment consisted of: IL-2 10MIU/m2, sc x 3/week, weeks 1-4, IFN-a 6MIU/m2, SC once a week, weeks 1-4 and 10MIU/m2 x 3/week, weeks 5-7, 5-FU 600mg/m2 and VBL 6mg/m2, iv bolus, weeks 5 and 8.

Results: In a median follow-up of 34 months, 58 pts were evaluated for tumor response. The other 4 were not included because of intolerance to treatment (3) and severe allergy (1). Response to immunochemotherapy: CR 4 pts (7%) for 26, 34, 51, 56 months, PR in 14 pts (24%) for a median of 14 months (4-48) and SD 20 pts (34%) for a median of 9 months (3-56). 7 pts (5 PR and 2 SD) underwent complete resection of residual tumor. 5 remained alive NED for 27, 32, 36, 42, 48 months. Therefore, 9 patients (16%) achieved long-term complete response for a median of 36 months from start of treatment. Three-year survival for the entire group was CR, PR, SD and PD pts: 30%, 100%, 30%, 40% and 0%, respectively. Three-year survival for CR patients and those who underwent resection of residual disease after immunochemotherapy (total 11 pts) was 88%. Side effects: flu-like symptoms, nausea, headache and depression.

Conclusion: Immunochemotherapy is effective and tolerated in pts with MRCC. Surgical intervention for resection of residual disease is justified.

843 POSTER

Modulation of TNF-alpha effects in presence of anti-CD45 and anti-CD95 antibodies in hematological cell lines

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TNF-alpha shows diverse effects on hematopoietic cells, suppress or stimulated of growth of several haematological cell lines. Based on this, evaluation of role cell surface associated antigens, partly members of TNF receptor superfamily, following cell death process was analyzed in our study.

Raji (malignant B-cell lymphoma) and PC (originally developed at Institute of Oncology in Sremska Kamenica, from MDS patients) cell line were incubated with and without TNF-alpha in final concentration of 500 and 1000 pg/ml of culture medium for short time period (30 min) in the presence of anti-CD45 and anti-CD95 monoclonal antibodies. The apoptotic and necrotic form of cell death were determined after duration of 2, 4, 6, and 24h by flow cytometric analysis (Becton Dickinson) after propidijum iodide and anexin V staining as recommendeded by the kit manufacturer (Pharmingen). Before and after treatment cell membrane antigenes expression were detected on gated cell population exluded debris.

The results showed that in comparison with untreated cells, TNF-alpha induced significantly increase in apoptotic and necrotic forms of cell death on Raji and PC cells. Apoptotic form of cell death, induced by TNF-alpha on PC cells pre-labeled with anti-CD95 MoAb, correlated with TNF-alpha effects alone at the same points, while cell death were significantly decreased after 24 h. Contrary to this, TNF-alpha shows maximal effects on Raji cells in comparison to controls after 2h with relatively constant effects analyzed after 8 and 24 h. TNF-alpha induced maximal necrotic forms of cell death between 6 and 8h on PC cells, while on Raji cells after 24h. TNF-alpha in a dose-dependent manner significantly decrease membrane expression on Raji and PC cells. Further analyses shows that antigen expression did not correlated with apoptotic form of cell death process in our two cell lines. Decrease of antigen expression for some molecules, partly TNF receptor superfamily members, after TNF-alpha treatment suggested their in-effectively for induction of apoptotic process, but their participation for transduction and modulation of death signals with different effects in examined cell lines.

844 POSTER

Determination of TNF alpha in supernates of stimulated PBL from cancer patients by two methods from one sample

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TNF-alpha is a cytokines whose level is determined in sera or in supernates of stimulated PBL of cancer patients during different treatment protocols. Aside from very specific ELISA assay for determination of TNF-alpha concentration, bioassay is the most common method for TNF alpha determination.

We determined TNF alpha in the supernates from 48h in-vitro LPS-stimulated PBL from malignant melanoma and breast cancer patients by a bioassay using L-929 TNF-sensitive cell line, at concentration 2.5 x 106/ml in 96 micro well flat-bottom plates. The standard curve was obtained with

known concentration of TNF-alpha (USA, Sigma) from 10 pg/ml to 1000 pg/ml and used for calculation of TNF-alpha concentration in the evaluated samples. The plates were incubated for 22 h at 370C with 5% CO2 in humid atmosphere, after which 10 ml/ml of actinomycin-D (final concentration of 0.01 mg/ml) was added for additional 2h. The supernates (0.1 ml) from previously stimulated PBL of cancer patients with LPS were added to the L-929 seeded plates and incubated during next 24 h. TNF-alpha was determined using microtiter plate reader Behringer EL-311 for bouth assays. The innovation presented in this work is in the possibility to determined TNF-alpha concentration from one sample by two different assays. In the one assay TNF-alpha concentration was obtained by analysing LDH-release from TNF-alpha killed L-929 cells. In the second assay after supernates was removed, the viable L-929 cells in the left monolaer were estimated by trichloroacetic acid and by methyl blue.

Using mathematics model for analyses of TNF alpha by the standard curve we obtained high correlation ratio for data determined by the two assays.

845 POSTER

Some vitiligo patients possessed functionally active immunotoxicity to melanoma Fem-x cells

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Purpose: The frequency of patients with vitiligo, or with melanoma, or healthy people, who possess an enhancement in the cytotoxic action of their immunocompetent cells to melanoma cells in vitro was evaluated.

Methods: Target cells (T) were human melanoma cell line, Fem-x (HLA-A2;B15,18+) and human cervix carcinoma HeLa cell line ((HLA-A1,9; B5,12+) which were used as control cells. Seven thousand of target cells were mixed with peripheral blood mononuclear cells (PBMC) as effectors (E), in E:T ratios up to 20:1in 15% of healthy AB+ fresh human serum (in order to asses only cellular immunotoxicity), and in 15% of serum of person whose immunnotoxicity was examined. For determination of target cell survival MTT test was used 24h later. The experiments were not done in the presence of complement inactivated serum because we determined that heating of examined sera for complement inactivation at 56C activated that some cytotoxic factors to melanoma cells.

Results: In the presence of healthy AB serum the enhanced PBMC cytotoxic activity to Fem-x in relation to HeLa cells, in 3/5 vitiligo, 0/4 melanoma patients and in 6/11 examined healthy sera was found. The additional enhancement of the PBMC toxicity was observed in 4/5vitiligo, 0/5 melanoma patients and 5/9 healthy people when the test was performed in their own serum in relation to the data obtained in control AB serum. The absence, or very low cytotoxic activity of PBMC was observed in 2/5 melanoma patients before chemotherapy. Moreover, some growth-stimulating activity of their PBMC under (E:T= 5:1 and 10:1) and of their own serum was found.

Conclusion: The comparison of the intensity of cellular and humoral immuno-dependent toxicity to melanoma and non-melanoma cells lines in melanoma and vitiligo patients and in healthy people was done. Preliminary results indicate that functionally active immunity special for melanoma cells is found more frequently in vitiligo patients, than in healthy people, or in patients with melanoma. The presence of antibodies to melanoma cells that could kill melanoma cells by ADCC action is also found in vitiligo patients. These findings deserve serous consideration regarding the development of adoptive imunotherapy based on the proper use of purified immune component from vitiligo patients in fight against melanoma.

Vaccination

846 POSTER

Co-vaccination with dendritic cells augmented suppressive effect on tumor growth by DNA vaccination targeting MUC1 tumor antigen

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Purpose: DNA vaccines have been reported to be beneficial for maintaining high levels of the antigenic protein expression and for eliciting strong anti-tumor immunity in vivo. However, suppressive effects by this treatment on tumor growth in tumor-bearing animals that had been reported are not satisfactory. We co-vaccinated with dendritic cells (DC) and MUC1 DNA in mice and assessed the ability of the DCs to enhance anti-tumor immunity.

Methods: C57/BL6 mice were vaccinated with expression vectors containing MUC1DNA after tumor challenge with MUC1 DNA-transduced tumor cells. In some mice, syngeneic DCs were inoculated simultaneously with DNA vaccination at the same site. The size of the tumor was monitored after challenge.

Results: The MUC1-transfectants grew in mice receiving vaccination with MUC1 DNA or control DNA, and all of the mice died within 6 weeks after tumor challenge. In contrast, tumor growth of MUC1-transfectants was markedly suppressed in mice receiving both MUC1 DNA and DC vaccination, and survival of these mice was significantly prolonged. Cell-mediated immunity to MUC1 was found to be enhanced in mice vaccinated with both MUC1 DNA and DCs compared to that in mice vaccinated with MUC1 DNA without DC inoculation.

Conclusion: DCs were suggested to augment anti-tumor immunity elicited by DNA vaccination, resulting in suppression of tumor growth in tumor-bearing mice. This animal model is useful for the development of a DNA vaccine for anti-cancer immunotherapy.

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Mouse model of human papillomavirus-associated tumors with downregulated MHC class i expression

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Purpose: Infection with human papillomaviruses (HPVs) can result in the development of both benign and malignant tumors, cervical carcinoma (CC) being the most serious disease associated with HPVs. However, many infections with HPVs are asymptomatic and transient and more than half of untreated mild precancerous lesions regresses spontaneously. Immune surveillance seems to be implicated in elimination of HPV-infected cells. One of the mechanisms enabling the escape to host immune system is the reduction of major histocompatibility complex class I (MHC-I) surface expression that has been recorded in more than 70% of CC patients. Therefore, the relevant animal model is necessary for development of therapeutic anti-HPV vaccines.

Methods and results: To evaluate the efficiency of experimental anti-HPV vaccines, TC-1 cell line has been prepared by transformation of primary C57Bl/6 mouse lung cells with HPV16 E6/E7 and activated H-ras oncogenes (Lin et al., Cancer Res. 1996; 56: 21-26). TC-1 cells are highly MHC-I positive. These cells were inoculated into mice preimmunized with E7 gene-based DNA vaccine and from tumors developed in some animals cell lines with downregulated MHC-I surface expression were isolated. When the expression of some components of antigen processing machinery (LMP-2, TAP-1, and TAP-2) was tested, reduced TAP-1 production was demonstrated in cell lines with downregulated MHC-I expression. In vitro treatment with IFN- resulted in upregulation of MHC-I antigens, but after IFN-gamma removal the MHC-I expression was again downregulated. The upregulation of MHC-I was also achieved in vivo. The increased immunoresistance of TC-1-derived cell lines with reduced MHC-I expression was demonstrated in animals immunized with plasmids containing modified E7 genes.

Conclusions: The model presented extends the possibilities of testing the therapeutic anti-HPV vaccines in settings more relevant to clinical requirements.