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.

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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.

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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