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.

847 POSTER

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.