Experimental Design: Patients with common human cancers underwent genetic analysis for suitable mutations in *ras* and p53. 39 patients were enrolled and vaccinated at least once. Patients had 17-mer peptides custom synthesized to their corresponding mutations. Baseline *in-vitro* immunological assays were performed to assess CTL response against mutant peptide-pulsed target cells and interferon-γ(IFN-γ) release from lymphocytes primed with mutant-peptide. Peripheral blood mononuclear cells were harvested and pulsed with patient's corresponding mutant-specific peptides, irradiated, and used for intravenous immunization schedule on days 0, 21, 77, and 133. Patients were followed for CTL, interferon-γ, IL-2, IL-5 and GM-CSF responses. They were also followed for signs of treatment-related toxicity and tumor response or progression.

Results: Toxicity potentially attributed to vaccination did not exceed grade I. Ten of 35 (29%) patients evaluated had a detectable cellular immune response against mutant p53 or ras and two patients had a positive CTL assay at baseline. Positive responses to IFN-γoccurred in twelve patients (34%) after vaccination, whereas six patients had positive IFN-γreaction to mutant peptides prior to vaccination. Of 27 patients with evident disease five had a period of stable disease, but all progressed eventually. Detectable cytotoxic lymphocytes or a positive IFN-γ release, but not IL-5 release, after peptide stimulation with mutant p53- or ras were associated with prolonged median survival. A positive IFN-γ response was associated with longer survival in multivariate analysis.

Conclusions: Custom made peptide vaccination is feasible without relevant acute or delayed toxicity. Pre-vaccination cellular immunity may exist and CTL and cytokine responses specific to a given mutation can be induced or enhanced. Evidence of cellular immunity to mutant p53 and K-ras oncopeptides was associated with longer survival.

987 POSTER

DNA encoding a Pan-MHC class II peptide analogue augmented antigen-specific cellular immunity and suppressive effects on tumor growth elicited by DNA vaccine immunotherapy

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Background: Activation of both helper and cytotoxic T cell function are required for eliciting a potent anti-tumor immunity capable of suppressing tumor progression by cancer immnotherapy. We previously reported that a sixteen-amino acid peptide analogue derived from pigeon cytochrome c (Pan-IA peptide) can bind a broad range of MHC class II types and activate the helper T function in mice. We addressed whether DNA encoding Pan-IA peptide can enhance the efficacy of tumor suppression by DNA vaccine targeting tumor antigens.

Material and methods: Pan-IA DNA was injected with DNA encoding a model antigen, ovalbumin (OVA) into C57BL/6 mice intramuscularly, thereafter spleen cells from these mice were examined for their proliferative responses and cytotoxic activities in response to OVA-expressing targets. To test therapeutic efficacy of the combined vaccines, E.G7 (OVA-expressing) tumor-bearing mice were treated with OVA and Pan-IA DNA vaccination.

Results: The specific proliferative response and cytotoxic activity were induced in mice vaccinated with both OVA and Pan-IA DNA, but not in those vaccinated with OVA DNA alone or control DNA plus Pan-IA DNA. Tumor growth of E.G7 cells was suppressed only by combined vaccination with OVA and Pan-IA DNA, and 5 of the 9 mice that received this combination completely eradicated the tumors. Both CD4- and CD8-positive splenic T cells from the mice receiving the combined vaccination showed proliferative responses to OVA-expressing cells, but only CD8-positive T cells were responsible for killing the targets in the effector phase. An immunofluorescent study showed that CD8-positive T cells commonly infiltrated the tumors of vaccinated mice. A distinctive feature of the co-vaccinated mice was that an increased number of CD4-positive T cells infiltrated the tumors.

Conclusions: The data suggest that Pan-IA DNA can augment antigen-specific cytotoxic activity of CD8-positive T lymphocytes via activation of the helper T function in DNA-vaccinated hosts. This animal model may contribute to the development of therapeutic DNA vaccines against cancer.

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Preventive vaccination of mice with human melanoma cells injected subcutaneously into encapsulated polyacrylamide gel

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Background: High degree of some melanocytic human and mouse antigens homology, presence of minor xenoantigens and species specificity are the main features in favour of xenogenic vaccination. In our studies, the vaccinating material was injected subcutaneous (s/c) into preliminary implanted encapsulated polyacrylamide gel (PAAG). The use of this approach affords effective protection of the xenogenic vaccine from rapid elimination by NK-cells.

Experimental goal: Elaboration of a method for xenogenic vaccination using a model of mouse melanoma -16.

Materials and methods: Intact mice (BDF) were injected s/c with 0.5 ml PAAG. After 3 to 6 weeks, melanoma cells (SKMEL-28 or cells isolated from surgical material) ($0.25E10^6$ $4,0E10^6$ cells/animal) were introduced into the gel. Six weeks thereafter melanoma -16 cells ($0.062E10^6$-$1.5E10^6$ cells/animal) were inoculated s/c into these mice. The melanoma B16 growth parameters and the cytotoxic activity of mouse splenocytes against B-16 cells were examined. Continuous control over the state of the gel, tumor nodes and animal lungs was made.

Results: It was found that the vaccination ($2E10^6$ cells/animal) inhibited melanoma B16 growth (inoculating B16 dose - $0.5E10^6$ cells/animal) by more than 70% for 2.0-2.5 weeks. An increase in the vaccinating dose enhanced this effect at later stages of B-16 development. For inoculating dose of B16 $0.062E10^6$ cells/animal, the vaccination prevents the melanoma growth in 72% of animals. The cytotoxic activity of splenocytes begins to increase from 2nd week following the vaccination, and remained at 50% level during 12 weeks.

Conclusion: Preventive xenogenic vaccination with subcutaneously injected and naturally encapsulated PAAG proved to be highly effective in melanoma growth prevention in mouse model.

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Identification of highly active, cancer specific promoters for use in targeted gene therapy of small cell lung cancer

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To identify highly and specifically expressed genes in small cell lung cancer (SCLC) we have used the DNA microarray technology. We analysed the expression of 12.000 genes in 21 SCLC cell lines and 6 xenografted tumors thereof and compared to the expression in 17 normal representative tissues. We have identified a number of genes with high expression in SCLC and low or no expression in all or most normal tissues. Only a few of these genes were previously known to be highly expressed in SCLC. Most of these genes are also expressed at similar levels in the published array data of 6 ressected SCLC tumors (Bhattacharjee et al., 2001, *PNAS, USA* 98:13790) demonstrating that the high expression is not an artefact due to establishment or propagation of cell lines.

Some of the expressed genes are known to be highly expressed in cancer cells due to hypo- or hyper methylation, such as the MAGEs or due to amplification, such as the myc family and are therefore not candidates for cancer specific regulation of expression. Several of the genes identified as highly expressed reflect the neuroendocrine origin of the cancers, such as INSM1, PTTG1, NCAM1, UCHL1 and ASCL1, which are known to be regulated in tissue and developmental manner by regulatory elements. These are therefore the most potential candidates to clone and test for cancer specific expression. However, there is a number of other genes known to be highly and specifically expressed in a variety of cancers such as EHZ2, STK12, KIAA0101, KIF14 and HSU79266. These genes are therefore candidates, but need further characterization.