Gene therapy

1275 POSTER

PRELIMINARY CLINICAL RESULTS OF ACTIVE IMMUNIZATION WITH INTERLEUKIN-4 GENE TRANSFECTED ALLOGENEIC MELANOMA CELLS IN METASTATIC MELANOMA PATIENTS

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From May 1994 to February 1995 five HLA-A2 patients affected with melanoma metastases were treated with active immunization with IL4 transfected, irradiated, HLA-A2 + allogeneic melanoma cells. They were injected with 50 × 10^6 cells at day 1, 13, 26, 55. Two patients received 4 vaccinations, 1 patient 3 vaccinations and 2 patients (actually not evaluables) were injected 2 times. According to the WHO toxicity grading 2 patients presented grade 2 local toxicity and 3 patients had grade 1. No systemic toxicity was recorded. No evidence of clinical response was obtained. At the present, 4 patients are alive with disease and 1 patient is alive without disease after surgical excision of a cutaneous metastasis.

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BIOLOGICAL CHARACTERISTICS OF SMALL CELL LUNG CANCER (SCLC) CELLS TRANSFECTED WITH HUMAN IL-2 GENE

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Aim of this study was the characterization of SCLC lines genetically engineered to express the immunostimulatory cytokine IL-2. Preliminarily, we examined by immunofluorescence and RT-PCR the expression of IL-2 receptor on 10 different SCLC lines to evaluate possible effects of IL-2 on the tumour cells. Only one SCLC cell line (GLC1) out of 10 tested expressed IL-2R α, β and γ chain while the others were negative. Human IL-2 gene was cloned by RT-PCR and inserted into the RSV.5 expression vector containing the neo resistance gene. By electroporation or lyposome-mediated transfection we have selected stable transfectants of 4 different SCLC lines (GLC1, N592, NCI-H69 and NCI-H164) secreting 25–200 U/ml of biologically active IL-2 in the supernatant. All IL-2 transfected tumour cell lines, including the IL-2R+ GLC1, displayed an "in vitro" proliferative potential (by MTT proliferation assay) similar to that of untransfected cell lines. Further analyses of the tumourigenic potential in nude mice are in progress. IL-2 transfected cells were able to stimulate the proliferation and to induce anti-tumour cytotoxic activity in peripheral blood lymphocytes.

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TRANSDUCTION AND SELECTION OF MURINE TUMOR CELLS ENGINEERED TO EXPRESS THE INTERLEUKIN 2 (IL-2) AND HERPES SIMPLEX VIRUS THYMIDINE KINASE (HSVTK) GENES

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One of the principal mechanisms by which the immune system fails to reject a tumor is a lack of cytotoxic response activation. In order to circumvent this problem, we have genetically engineered murine colorectal carcinoma cells to actively express the IL-2 gene, introduced using three different retroviral vectors (DCTK-IL2, LNCX-IL2, LXSN-IL2). These three vectors also carried the neoR gene, which confers resistance to *in vitro* treatment with a neomycin analog (G418). We evaluated the effect of different selection methods on the final gene expression. Previous experiences demonstrated that, out of the three retroviral vectors with the IL-2 gene, one of them (DCTK-IL2) was very effective in stimulating a systemic specific antitumor response, but an inappropriate local response against the immunization dose. In order to control the growth of tumor cells transduced with the DCTK-IL2 vector injected as active immunization, these cells were further transduced with a retroviral vector carrying the neo gene and a "suicidal" gene, which codifies for the HSVTK. For these cells with a double transduction, we designed two selection methods (initially with high doses of G418 or HAT followed by G418) in order to identify the subclones which had the most favorable expression of both the IL-2 and HSVTK genes.

IL-2 production increases both with increasing *in vitro* concentrations, and with duration of G418 selection. Assayed with a commercial IL-2 ELISA, the DCTK-IL2 vector produced the lowest IL-2 levels *in vitro* (2 U/10^6/24 h), whereas the other two retroviral vectors produced similar levels of IL-2 (18 U/10^6/24 h). Cells with a double transduction were able to successfully tolerate higher levels of G418 in the culture (up to 1.6 mg/ml) due to the presence of two copies of the neoR gene. Both methods of selection of these cells (high dose G418 or HAT) produced cell populations sensible to GCV *in vitro*. We obtained 55 subclones from different moments of the ongoing cell selection, and the clones with most favorable expression of both genes (IL-2 and HSVTK) were expanded for further *in vivo* studies. In conclusion, we have shown that both the level and duration of selection of retrovirally transduced cells have an important impact on gene expression, and that it is possible to select cells with a double transduction with two vectors carrying the neoR gene. The most favourable cell clones will be used as active antitumoral immunization (vaccines) in further gene therapy experimental studies in Balb/c mice.