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IMMUNO-GENE THERAPY OF RAT GLIOMA WITH TGF-β2 ANTISENSE AND IL-2 GENE MODIFIED TUMOR CELLS

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We evaluated the efficacy of immuno-gene therapy in the rat 9L glioma tumor model. Like many human gliomas, 9L cells secrete TGF-β2, an immunosuppressive agent which inhibits normal T cell activation. We genetically modified 9L cells to secrete human IL-2 with the LNCX-hIL2 retroviral vector resulting in secretion of 10^3 units IL-2/10^6 cells/24 hr. In addition, both the parental and IL-2 transduced 9L cells were transfected with a TGF-β2 antisense plasmid vector to inhibit TGF-β2 expression. 5 x 10^3 live parental tumor cells were implanted into the forebrain of the animals and immunizations were begun 4 days later. The animals were immunized subcutaneously 4 times on a twice a week schedule with 2.5 x 10^5 irradiated cells from the following treatment groups: 1) control vector modified 9L cells; 2) 9L cells transduced with IL-2; 3) 9L cells transfected with the TGF-β2 antisense vector; and 4) 9L cells transduced with IL-2 and transfected with the TGF-β2 antisense vector. The tumor free survival rate 9 weeks post tumor implantation was 2/10 and 3/10 for the groups immunized with parental 9L and 9L IL-2 transduced cells respectively. Transfection of both 9L cells (6/6) and 9L IL-2 transduced cells (7/7) with the TGF-β2 antisense vector resulted in a statistically significant increase ($p < 0.01$) in the number of tumor free animals 9 weeks post tumor implantation. These data indicate that inhibition of TGF-β2 expression significantly increases the efficacy of tumor cell vaccines in the 9L glioma model. Our findings suggest that strategies to inhibit the expression of immunosuppressive factors may be important in the development of future immuno-gene therapies.

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TRANSFER of the METALLOTHIONEIN GENE to CD34+ CELLS USING an ORIGINAL FRIEND VIRUS DERIVED CONSTRUCT CONFERS RESISTANCE to CIS-PLATINIUM DERIVATIVES and to ALKYLATING AGENTS

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Only significant technological improvements will allow for therapeutic benefit using gene transfer and therapy approaches. We have designed an original retrovirus vector based on Friend-MuLV (Fr-MuLV) FB29-strain selected according to both high infectivity and haematopoietic tropism in mouse. A Neo^Rgene carrying -construct producing viral titers over 10^7 cfu/ml has first been used.

Our primary goal consisted in the demonstration of efficient transduction of haemopoietic progenitors of human origin. We first settled reproducible in vitro growth conditions of CD34+ cells in long term cultures ; established onto xenogeneic stromal cells ; eventually engineered to resist G418-selection. Infection conditions have been optimized comparing various procedures onto the **same source** of cells. We aimed at : 1st) using as few growth factors and stimulatory events as possible to spare pluripotentiality of progenitors ; and 2nd) achieving infection exposing cells to viral supernatant to avoid co-cultivation.

These cultures have been maintained during a two months period during which sequential assessment for retroviral transduction has been performed using either pharmaco-biological (CFU-GEMM +/- G418 selection) or molecular (PCR onto individual GFs-expanded colonies) characterization. We have been able to demonstrate : 1st) an initial transduction efficiency of 50 to 90% CFU-GEMM generating cells following several infection cycles with viral supernatant ; 2nd) efficient transduction of haemopoietic progenitors capable of generating both long-term in vitro cultures of 60 days in the presence of G418 and further growth under the form of G418-resistant CFU-GEMM seeded at day 60 ; where virus integration could be further evidenced on individual colonies by PCR. In our hands, transduction efficiency was critically dependent on viral titers ; reproducible data could be obtained with our stable producer of 10^7 cfu/ml, but not with titers of 10^5 cfu/ml. We are currently evaluating the efficiency of our Fr-MuLV-construct in comparison with identified efficient retrovirus vectors.

The next step consisted in introducing a gene of interest into CD34+ PBMSC (peripheral blood mobilized stem cells). We chose to work with a gene confering resistance to cytotoxic drugs to achieve prevention of myeloablation following intensive chemotherapy regimens using the Metallothioneine IIA gene ; overexpression of the latter has been shown to confer resistance to both Platinium derivatives and to alkalating agents. A full genomic clone of the Metallothioneine IIA gene has been introduced in our Friend-virus vector either in sens orientation ; or in reverse orientation to spare the genomic structure of the construct following tranduction of target cells. High producing clones have been selected following transfection into the amphotropic packaging cell line Psi-CRIP on the basis of molecular transmission (PCR) ; and submitting infected cells to increasing doses of selective medium.

We have been able to demonstrate significant resistance of both 3T3-cells and human CD34+ PBMSC. With a high producer including the transgene into sens orientation, the IC50 of 3T3 shifted from 5-20 µM to more than 150 µM of Cisplatinium (CP) ; control cells were both 3T3 cells and Neo-infected cells. The pourcentage of viable cells in the presence of 75µM CP, was null for either of the controls and over 80% for MTIIA infected cells. Significant resistance of CD34+ PBMSC could be demonstrated following infection as assessed by measuring both the pourcentage of viable cells and the number of CFU-GEMM in the presence of increasing concentrations of CP. We are currently analyzing the spectrum of induced resistance to myeloablative alkalating agents.

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ADENOVIRUS-MEDIATED GENE TRANSFER OF THE HSV-THYMIDINE KINASE GENE FOR THE TREATMENT OF PRIMARY CNS MALIGNANCIES

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Primary CNS malignancies in adults are nearly uniformly fatal despite improvements in diagnostic and surgical techniques and advances in radiation therapy. Conventional chemotherapy provides only minimal benefits. We have employed adenovirus-mediated transfer of the drug susceptibility gene herpes virus thymidine kinase (HSV-TK) into primary brain tumors to achieve significant tumor reduction in murine models of human gliomas. Intra-tumor injection of recombinant adenovirus expressing HSV-TK (H5.010RSV-TK), followed by intravenous ganciclovir results in tumor regression in several rodent and human brain tumor cell lines in animal model experiments. The extent of tumor regression is dependent on the size of the tumor, the amount of virus administered, and the duration of ganciclovir treatment. In anticipation of clinical application of these findings, we have extensively studied the toxicity of H5.010RSV-TK in rats and in non-human primates. This recombinant adenovirus, when administered into the CNS of normal animals, results in mild dose-dependent toxicity. MRI and histologic studies show focal inflammatory effects that resolve over a period of several weeks. All animals tolerated the treatment well up to the maximally administered doses of 5.7×10^9 pfu in rats, and 1×10^{11} pfu in rhesus monkeys.

We have designed a phase I clinical trial to test the safety and efficacy of this approach in patients with refractory gliomas. The design of this clinical trial will be discussed.

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In vivo gene therapy of metastatic melanoma using the HSV*tk* gene by co-activation of anti-tumour immunity.

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We are interested in developing high efficiency, targeted gene therapy delivery systems for the treatment of established tumours *in vivo*. Using melanoma as a model, we have shown that the murine tyrosinase promoter can be used to express a range of heterologous genes specifically in both human and murine cells of melanocytic origin. Rather surprisingly, multiple intravenous administrations of high titre retroviral supernatant encoding Tyr-HSV*tk* reduced the number of lung metastases of B16 melanoma in C57/BL mice treated with ganciclovir by up to 90% compared to controls. This reduction was not observed in T cell immunodeficient mice. In addition, a partial anti tumour immune response was generated following *in vivo* killing of established tumour deposits with ganciclovir, suggesting a role for the immune system in the observed effects. Using reverse transcriptase PCR, we have shown that, as tumours are killed *in vivo* by ganciclovir, *de novo* expression of several different cytokines can be detected, including IL-2, IFN-gamma, IL-6 and GM-CSF within the tumour. Therefore, we have now prepared vectors in which the HSV*tk* gene is co-expressed with a series of immunomodulatory genes with the aim of augmenting this anti-tumour immunity. Co-expression of *IL-2* and HSV*tk*, even in only a fraction of tumour cells, leads to a significant reduction in the growth rates of tumours growing *in vivo*. In addition, animals in which 10% of the cells of the primary tumour expressed both *IL-2* and *tk* develop increased protection against a subsequent challenge with parental cells compared to animals in which 10% of the cells of the primary tumour expressed either gene alone. The immune-mediated mechanisms underlying the action of these vectors, and their potential for direct *in vivo* gene therapy of tumours, will be discussed.