initial stocks obtained by transfection, wild-type MVM still appeared during serial infections. To avoid the recombination events between vector DNA and helper sequences, responsible for these contamination with wild type virus, we removed all homology between helper and vector sequences. We based these sequence modifications on the study and sequencing of spontaneous extremely small defective virus, and on the degeneration of DNA sequences with no alteration of amino acid sequences. We thus setup a rapid and simple method that should be easily upscalable, for the production and purification of high titer vectors suitable for in vivo testing of the therapeutic efficiency of recMVM vectors against tumors. In a strategy of immunotherapy of cancer, in vitro and preliminary in vivo results have been obtained by using them to transfer the IL-2 cDNA.

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Genetically modified melanoma cells as cancer vaccines

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Gene therapy approaches for the successful combat of cancer include several conceptually different strategies: (i) enhancement of the tumor's immunogenicity; (ii) modification of the host immune system; (iii) modification of other host tissues, e.g., by transfer of drug resistance genes into hemopoietic progenitor cells; (iv) introduction of corrective genes (e.g., wild-type p53) into tumors; (v) transfer of enzymes for prodrug therapy.

In the case of skin cancer, most gene therapy trials are conducted in patients with disseminated melanoma using tumor cells whose immunogenicity has been augmented by transfection with genes encoding cytokines (e.g., IL-2, IL-7, GM-CSF) and/or costimulatory molecules (e.g., CD80).

We and others have shown (i) that highly tumorigenic mouse melanoma cell lines lose their tumorigenicity upon transfection with IL-2, (ii) that mice injected with IL-2-transduced melanoma cells are protected when challenged with wild-type tumor cells, and (iii) that administration of IL-2-transfected melanoma cells into mice can induce elimination of preexisting cancer cell deposits. Based on these encouraging results, we have used IL-2-based, autologous human melanoma vaccines in a phase I trial in patients with stage IV melanoma. The vaccines' recipients did not show any overt signs of systemic toxicity and some of them developed positive delayed-type hypersensitivity (DTH) reactions to autologous melanoma cells after 2-3 vaccinations. 3/15 patients experienced a prolonged stabilization of their disease but, ultimately, all vaccine recipients succumbed to their disease.

Recent evidence indicates that cytokine-based cancer vaccines exert their protective effect in experimental animals not by a direct stimulatory effect on T cells but rather by the initiation of inflammatory events leading to the presentation of vaccine fragments by host-derived antigen-presenting cells. Assuming that this cross-priming phenomenon would also be operative in cytokine-based human cancer cell vaccines, we have recently begun to test the safety and tolerability as well as the immunological efficacy of IL-2-transfected allogeneic melanoma cells in patients with stage IV disease. Their ultimate value for therapeutic purposes remains to be determined, in a controlled fashion, in patients with less advanced melanoma.

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Anti-tumoral effects following Ad-mediated delivery of angiogenesis inhibitors

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Angiostatin is an inhibitor of angiogenesis secreted by tumors, driving metastasis into a dormant state. We have constructed an E1-deleted adenovirus that expresses the N-terminal fragment (amino-acids 1–333) of human plasminogen, including its preactivation peptide and kringles 1 to 3 (AdK3). AdK3-infected endothelial cells showed a marked, dose dependent arrest in proliferation in vitro. A single intratumoral injection of AdK3 was shown to dramatically inhibit primary tumor growth in two preestablished xenograft murine models. This inhibitory effect on tumor growth was tightly correlated with a markedly decreased vascularization within, and at the vicinity of the tumors, and a 10-fold increase in tumor cell apoptosis.

We have also assessed in mice the antitumoral effects that specifically follow the administration of AdmATF, an E1-deleted adenovirus that expresses a secretable antagonist of urokinase (uPA) which binds to its cell surface receptor (uPAR). Using different murine tumor models, we have shown that the intratumoral expression of ATF inhibits primary tumor growth and interferes with tumor cell dissemination, effects correlated with a marked inhibition of angiogenesis within and at the vicinity of the tumor mass. Finally, we have also shown that a systemic administration of the virus could protect against subsequent tumor challenge.

Angiostatic therapy using recombinant adenoviruses is plausible and efficient; nevertheless, maximal clinical benefits will require improved vectors able to sustain transgene expression.

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Modulation of antigen presenting cells-tumor cells interactions

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One of the most important finding in immunology is the discovery of peptides as the entities that bind MHC to signal self identity on the cell surface.

For the majority of neoplasias the identity of their tumor antigens remains to be determined and even for melanoma it is clear that we know only a minority of all possible antigens that could be expressed by this tumor.

Therefore, a tumor vaccine should face two different situations depending whether the antigen is known or not.

In case of known antigen the current strategy consist of loading DC with the peptide or protein or of transfecting DC with the gene coding for the antigen. Our approach is to deliver the tumor antigen directly into APC through an oral vaccination performed with attenuated bacterial vectors, such to avoiding any ex-vivo manipulation of DC. A live attenuated aroA auxotrophic mutant of Salmonella typhimurium has been used as carrier for the pCMVb vector that contains b-gal gene under the control of the immediate early promoter of Cytomegalovirus (CMV). After three courses, at 15-days interval, mice developed a specific b-gal CTL response as well as antibodies response. Mice vaccinated with the Salmonella harbouring pCMVb, but not with plasmid less carrier, showed resistance to a challenge with a highly aggressive murine fibrosarcoma transduced with the b-gal gene that behaves operationally as a tumor-associated antigen. These experiments show that Salmonella-based DNA immunization allows to specifically target antigen expression in vivo to APC, and results in induction of efficient MHC-I and II-restricted anti-tumor immune responses.

Tumor cells likely present the entire repertoire of tumor associate antigen, either known or unknown, of a certain neoplasm. To create a cellular vaccine that should favour direct, in vivo, tumor-DC interactions we transduced BALB/c-derived C-26 colon carcinoma cells with granulocyte-macrophage colony stimulating factor (GM-CSF) and CD40 ligand (CD40L) genes. DC infiltrating tumors producing GM-CSF and CD40L capture cellular antigens, likely through uptake of apoptotic bodies, and mature in situ to a stage suitable for antigen presentation to T cells. Thus, tumor cell-based vaccines engineered to favour the interaction with host DC can be considered.

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Abstract not received.