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Clinical Application of Immunostimulatory Gene Transfer

B. Gansbacher

INTRODUCTION

Despite significant progress in the treatment of leukaemias, non-Hodgkin's lymphomas and Hodgkin's lymphoma, there is still much improvement to be made with chemotherapy for solid tumours. This can be illustrated by the fact that, in 1991, approximately 165 000 Americans developed lung cancer and, despite receiving optimal therapy with chemotherapy or radiation therapy, 143 000 died the same year. Similar observations have been made for many other solid tumours. Chemotherapy and radiation therapy have improved survival rates significantly, but new therapeutic strategies are required.

In 1980, several groups began developing new concepts to treat cancer which were based on activation of the immune system. It was known that effector cell populations of the immune system can kill tumour cells *in vitro*. Lymphocytes, natural killer cells, macrophages or lymphokine activated killer (LAK) cells, when co-cultured in a test tube with tumour cells, will kill the cancer cells under certain conditions. Discussion rapidly took place regarding manipulation of the effector cells *in vivo*. The fact that some of the effector cells expressed interleukin-2 (IL-2) receptors led to several studies in which systemic IL-2 was used in an attempt to mobilise and activate them.

Over the last 10 years, IL-2 has been used extensively in both animal models and cancer patients; it has been injected intravenously into lymph nodes or directly into tumours. However, side-effects have been common, and the results unimpressive. Partly to compensate for the short-half life of IL-2, megadoses have been given. Several patients treated in this manner have required intensive care and some have died. By 1989, it was clear this was not the optimal method of stimulating effector cell populations *in vivo* and new modalities were needed. It was believed that cytokines were necessary at the site, where effector cells encounter the tumour, because they were known to drive the differentiation and development of functional abilities of relevant effector cells. By using retroviral vectors, DNA transfection, adenoviruses and lipofection, several groups introduced cytokines directly into tumour cells and in this way forced them to secrete the cytokine directly at the tumour site. The cytokines included IL-2, IL-3, IL-4, IL-6, IL-7, granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), tumour necrosis factor (TNF), and many others [1].

Several cytokines have induced rejection of primary tumours in murine models. The effects on the immune system, induced by various cytokines, were compared with each other. Despite the attempt by some investigators to claim that they had found

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the "magic bullet", contradictory data accumulated rapidly. For example, a number of promising reports were published on cytokines and tumour rejection: IL-2 was reported to induce rejection of an established murine bladder carcinoma [2], IL-4 induced rejection of an established renal carcinoma [3], IL-6 induced rejection of an established Lewis lung carcinoma [4], and the co-stimulatory molecule B7 induced rejection of a murine melanoma [5, 6]. However, other investigators failed to confirm these results and reported that similar or higher amounts of IL-2, IL-4 or B7 were not able to induce rejection of other, already established, tumours. These findings are not surprising considering that the murine tumours have different characteristics. Some express chemically-induced tumour-associated antigens, others are virally induced and many do not express tumour-associated antigens. Considering the natural make-up of human tumours, it can be predicted that murine tumours expressing chemically- or virally-induced antigens, not encountered in human cancer patients, will not be predictive for human cancer gene therapy trials.

FROM MICE TO MEN

The occasionally spectacular results seen in murine tumour models have formed the basis for the development of similar approaches in cancer patients. Before permission is given to use gene therapy in human cancer patients, experimental data are required by the Federal Drug Administration (FDA) and Recombinant DNA Advisory Committee (RAC). Although mouse models will not be predictive of clinical responses in human cancer patients, the federal agencies request that each group documents its expertise with gene transfer methodology, its ability to deal with safety-related problems and answers any relevant scientific questions.

Safety considerations have been essential in the development and evaluation of cytokine gene therapy. The first step was to eliminate the necessity to inject live engineered tumour cells by showing that irradiating tumour cells would not lead to loss of cytokine secretion. Another important issue in the evaluation prior to approval is the demonstration that there is no replication-competent helper viruses after transduction and irradiation of the transduced cells. During the transfection process, the recombinant virus could potentially recapture deleted structural genes and could revert to a replication-competent virus. Mus-duni cells are murine fibroblasts that are permissive for ecotropic and amphotropic viruses. By co-culturing transduced tumour cells for 6 weeks with Mus-duni cells, the generation of replication-competent virus by the transduction procedure or the radiation can be excluded.

The first step in developing clinical gene therapy protocols requires documentation that expression systems, such as retroviral vectors, are functional in human cells. Other important variables are duration of expression and biological activity of the secreted molecules. As in the murine models, IL-2 was the most frequently used molecule in the initial approaches. Our group investigated the effect of IL-2 secretion by tumour cells on human cytotoxic T-lymphocytes (CTLs) or LAK cell populations from a patient with renal cell carcinoma. IL-2 secretion by autologous tumour cells, used to restimulate the cells *in vitro*, increased the lytic activity of CTLs, but not of LAK cells [7]. Similar results were generated by other investigators in melanoma patients. Humoral and cellular anti-tumour responses were found predominantly in melanoma, the first human tumour where a CTL-defined tumour antigen was found [8]. This antigen was named melanoma-associated antigen E (MAGE1) and proved that CTL-defined tumour-associated epitopes exist [9]. The CTL precursor frequency was surprisingly high, ranging from 1 in 910 to 1 in 33 000 mononuclear cells. This implied that tumour-specific CTL did not have to be generated *de novo*, but were already present in the circulation of the cancer patients and might be clonally expanded if the appropriate stimulus were given. As a result of these findings, gene therapy was attempted in melanoma patients first.

Whether to use an allogeneic or autologous tumour vaccine is another important decision when developing gene therapy protocols. Establishing tumour-derived cells lines is sometimes feasible, but always cumbersome. In melanoma, it is possible to establish cell lines in approximately 30–40% of patients. If autologous tumour cells lines are used as a vaccine, cell lines must first be generated, the transduced cell lines characterised and then safety studies performed. The considerable time required to carry out these procedures poses difficulties when managing patients with metastatic disease. An additional option is to transduce primary tumour cells without selection [10]. However, there are no data to suggest that autologous transduced tumour cells are better than allogeneic human leucocyte antigen (HLA)-matched tumour cells for stimulation of tumour specific CTLs. There are data showing that tumour cells are not optimal for antigen presentation [11]. Usually, they are poorly immunogenic, can have defects in antigen presentation pathways, and do not express co-stimulatory signals. Some of them do not express major histocompatibility complex (MHC) class I molecules or secrete immunosuppressive factors, such as IL-10 or tumour growth factor-β.

Attempts are underway in several laboratories to use genetically-modified professional antigen-presenting cells, such as dendritic cells, for CTL restimulation. The rationale for using allogeneic cell lines matched for one HLA class I antigen is the evidence that HLA-A2 molecules can present shared immunogenic peptides to cytotoxic T-cell populations, that HLA-A2-restricted melanoma-specific CTLs can kill allogeneic HLA-A2-positive melanomas [12], and the belief that the alloantigens may serve as adjuvant to enable a stronger anti-tumour response.

HUMAN CLINICAL GENE THERAPY STUDIES

The field of the clinical application of immunostimulatory gene transfer has expanded dramatically. Approximately 50 centres worldwide use gene therapy approaches for cancer. The goal of all the immunostimulatory gene therapy protocols is activation and stimulation of T cells. Memory and specificity are prerequisites of this approach, and the killer cells generated must have the ability to travel from one tumour location to the next, specifically recognising and killing those tumour cells. T cells are the only cells characterised by memory and specificity within the body.

The first protocol approved by the federal government was a marker study conducted by Rosenberg's group in June 1988. Tumour-infiltrating lymphocytes were marked with neomycin resistance gene and the cells infused into patients with advanced cancer who were treated with cyclophosphamide and IL-2. Two years later, the group obtained permission to assess gene therapy in patients with advanced cancer, using tumour-infiltrating lymphocytes transduced with the gene coding for the TNF-α. Particular difficulty was encountered in generating vectors that would induce secretion of sufficient amounts of TNF-α. Soon after, studies began at the University of Pittsburgh, where patients with melanoma were treated with tumour-infiltrating lymphocytes transduced with either IL-2 or IL-4 cDNA.

Those initial studies revealed that it was very difficult to transduce human T-lymphocytes, and to generate sufficient numbers to reinfuse into patients. With this in mind, investigators focused on attempts to transduce tumour cells themselves and to force them to secrete cytokines needed by the T-lymphocytes. Again it was Rosenberg's group that was given permission in 1991 to immunise cancer patients using autologous cancer cells modified by the insertion of the gene for TNF-α. In a subsequent protocol, patients were immunised with autologous cancer cells modified by the insertion of the IL-2 gene. Nabel and colleagues were the first to use liposomes to transfect the HLA-B7 molecule into tumour cells. Injections were given directly into metastatic sites. 5 patients finished the study and one partial response was seen [13].

Subsequently, similar approaches were used in many centres. Brenner and colleagues inserted an IL-2 gene in autologous neuroblastoma cells for the treatment of relapsed or refractory neuroblastoma [14]. Gansbacher and colelagues were the first to use an allogeneic approach [15, 16]. In a pilot study, HLA-A2-matched patients with metastatic melanoma or renal cell carcinoma were immunised with HLA-A2-positive allogeneic melanoma cells or renal carcinoma cells secreting IL-2. Early in 1993, an alternative method of cytokine delivery to the tumour site was initiated by Lotze's group at the University of Pittsburgh. Autologous fibroblasts were transduced with the IL-4 gene, mixed with autologous tumour cells, and injected into melanoma patients [17]. In an attempt to induce differentiation and maturation of professional antigen-presenting cells, GM-CSF-transduced autologous tumour cells have ben used [18]. This was a phase I study of non-replicating autologous tumour cells injections using cells prepared with or without GM-CSF gene transduction in patients with metastatic renal cell carcinoma. As primary renal carcinomas themselves secrete GM-CSF in nanogram amounts, the study was split into two arms. One group was vaccinated with irradiated autologous tumour cells and the second injected with GM-CSF transduced autologous cells. Gamma interferon (IFN-γ) is being used at Duke University to stimulate the host antitumour response in a phase I trial of human IFN-γ transduced autologous tumour cells in patients with disseminated malignant melanoma. Another cancer where the IL-2 cDNA was introduced into autologous tumour cells is lung cancer. A phase I study, at the University of Miami, uses transfected cancer cells expressing the IL-2 gene product in limited stage small cell lung cancer.

Brain tumours have been another target. By introducing producer cell lines into the brain, only replicating cells are infected by the recombinant virus, while normal brain cells remain uninfected. Thymidine kinase (TK) induces phosphorylation of gancyclovir to a toxic metabolite. By using a producer cell line that generates a TK-containing virus, dividing cancer cells can be transduced with the TK gene, such that toxic metabolite is produced selectively. Several patients with gangliomas have been treated at the NCI and clinical responses have been observed in some.

Thus, a multitude of gene therapy trials have been started in end-stage cancer patients. All are phase I studies with the main focus on assessing toxicity.

CONCLUSION

Dramatic tumour responses in murine tumour models have prompted cytokine gene therapy trials in cancer patients. However, there are many reasons why the promising animal data may not translate into positive clinical results. First, it is known that some human tumours secrete cytokines; GM-CSF, IL-5, IL-6 for example. To date, no tumour has been found that secretes IL-2, so there is considerable promise in the use of IL-2. Second, mouse tumours have different characteristics to human tumours. They are artificially induced and kill the host in about 20 to 40 days, while human tumours arise spontaneously and have a different natural history with different growth behaviour. A breast carcinoma may take 4 to 6 years to reach a size of 1 cm^3 (approximately 10^9 cells). Third, several human tumours have been found that secrete immunosuppressive factors. For example, 80% of colon carcinomas secrete IL-10. Fourth, many tumours are poor antigen presenters or do not express MHC class I at all. This diversity can even be seen within the same tumour cell population. Thus, caution is warranted when considering the impressive antitumour responses observed in the murine tumour models in relation to the anticipated responses in patients.

Finally, tumour burden is an important consideration. A 10:1 ratio of effector to target cells is required to see significant killing *in vitro*. Since a tumour of 1 cm^3 already contains 10^9 cells, 10^{10} tumour-specific CTL would be required to eliminate all cancer cells. It is apparent from this estimate that the number of CTL required to eliminate bulky disease is not compatible with human life. Perhaps only in residual disease will such cytokine gene therapy be feasible.

The initial phase I cytokine gene therapy trials are unlikely to generate significant antitumour responses. Rather, they will serve as a tool to highlight the pitfalls of using these new expression systems. It is likely that the next generation of trials will be done in patients with less tumour burden and will be used to evaluate the full power of this approach. Although animal models will never accurately predict human responses, it is reasonable to request that investigators using this technology show first in murine models that they have the expertise to deal with these complex systems and that they have the know-how to evaluate this modality to exclude the generation of dangerous side-effects. Only then should carefully planned human trials be undertaken and evaluated critically.

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In situ Delivery of Suicide Genes for Cancer Treatment

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INTRODUCTION

THE TRADITIONAL treatments for cancer—surgery, radiation and chemotherapy–have shown only a modest increment in improved therapeutic outcomes during the past decade, and may be nearing their maximum effectiveness as therapy for many cancers. Clearly, new approaches are needed if better results are to be obtained for several tumour types. The past decade has seen early development of several biologically-based cancer treatment strategies, particularly immunotherapy, with great anticipation that these techniques will provide a fourth significant treatment option for cancer. Unfortunately, with a few notable exceptions, the results of biologically-based treatments for most cancers have been disappointing.

There has been a recent upsurge in excitement in this area because the initial clinical applications of gene therapy have rekindled the hope that biological treatments for cancer may finally become the effective weapon against cancer that has been so long anticipated. Two broadly-defined strategies for gene therapy of cancer are currently being explored. In one, which can be defined as an indirect or adjuvant approach, gene insertion is directed at enhancing the anti-tumour response of a patient. As outlined by other contributors to this volume, this strategy may include attempts to use gene insertion to improve the anti-tumour response of a patient's T-cells by modifying them directly, by introducing genes to render normal tissues (e.g. bone marrow) resistant to chemotherapy, or by genetically modifying the tumour in order to enhance its immunogenicity and induce an effective systemic anti-tumour immune response.

This report will discuss another strategy for gene therapy of cancer—the attempt to directly treat cancer by gene insertion. The power of gene transfer offers a unique opportunity to develop entirely new treatment strategies for cancer. However, many formidable obstacles will have to be overcome in order to make these approaches clinically useful.

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REVIEW

The approach to direct gene therapy for cancer has the basic premise that if exogenous genes could be successfully delivered to every cancer cell in a patient, then a whole range of new treatment options might become possible. For example, wild type tumour suppressor genes might be introduced into malig-