



Review

Cancer Immunogene Therapy

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Abstract. The establishment of cancer in a host involves at least two major events: the escape of tumor cells from normal growth control and their escape from immunological recognition. Because of this nature of their development, cancer cells seem to be predominately poorly immunogenic. In contrast to the previous idea that cancer cells express no recognizable antigens, recent progress in the identification and characterization of tumor antigens, as well as the expansion of knowledge on the cellular and molecular mechanisms of antigen recognition by the immune system, have raised the possibility of using immunotherapy to treat certain tumors. Information on these mechanisms has been obtained in three crucial areas: 1) the role of cytokines in the regulation of the immune response, 2) the molecular characterization of tumor antigens in both mouse and human tumors, and 3) the molecular mechanisms of T cell activation and antigen presentation. Such information has provided new insight into tumor immunology and immunotherapy. Furthermore, recombinant DNA technology allows for modification of the genome of mammalian cells for therapeutic purposes in several diseases. Several novel strategies have been developed to derive genetically modified tumor cells and use them as cellular vaccines to induce antitumor immunity in animal tumor models. This combined modality of genetically modified tumor cells and immunotherapy has been termed immunogene therapy of tumors. Crucial to this approach has been the ability to transfer into normal or neoplastic cells genes known to increase the immunogenicity of cells, which subsequently can be used to augment immune reactions in tumor-bearing mice or cancer patients. While there has been success in inducing antitumor immunity in some tumor models, there are difficulties and limitations in the application of these gene-modified tumor cells for the treatment of preexisting tumors. In this review, recent progress in cancer immunogene therapy is discussed.

Key words: immunotherapy; transfection; cytokine; costimulatory molecule.

Introduction

Despite successful local therapy, such as surgical excision or elimination with radiotherapy, many malignancies will recur locally or, more commonly, at a distant site. Thus, the prevention of metastasis remains a major focus of clinical oncology. Although many treatment modalities for cancer have been tested for an effective cure of cancer, only a limited number of pa-

tients have benefited from the currently available treatment. Chemotherapy is often the modality of choice for the treatment of an established metastasis, but its use is limited because of its toxicity and mode of activity against many common forms of cancer. Many of the cytotoxic agents are aimed at suppressing the process of cell division, which is not unique to cancer cells, resulting in the damage of normal cells and inducing many forms of severe toxicities. The most important

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aspect of cancer treatment is whether the treatment can induce specific damage or the death of cancer cells. In this respect, immunotherapy has been considered an attractive alternative.

A critical aspect of immunotherapy is based on the fact that experimental rodent tumor cells express antigens capable of eliciting specific and protective immune responses. Recent findings suggest that protein products of activated oncogenes, rearranged normal genes and overexpressed normal genes are potential targets as rejection antigens recognized by the host immune system. Because of the very weak antigenicity of these molecules, the therapeutic potential of immunotherapy depends on how the tumor cells can be manipulated to augment immunogenicity and stimulate the host immune system.

Strategies to enhance the recognition of tumor cells by the immune system can be classified in three categories: 1) those using tumor cells modified with cytokine genes, which are known to enhance tumor recognition or tumor rejection, 2) those aiming at the expression of co-stimulatory molecules on the surface of tumor cells, and 3) those based on the presentation of tumor antigens by antigen-presenting cells (APC). Treatment systems established in animal studies are now being tested in clinical settings and the results of these tests will determine the direction that immunogene therapy for cancer takes.

Cytokine Immunogene Therapy

As for the immune system, cytokines play an essential role in almost every step of the process leading to the recognition of antigens by T lymphocytes and the subsequent events that may result in tumor cell rejection or, even, the opposite effect, tumor growth promotion. The cytokines that could be involved in the regulation of tumor immunity are summarized in Table 1. Although some of these cytokines act in the immune response against cancer, the use of recombinant cytokines as a systemic therapy has produced discouraging results. This can be explained in part by the toxicity of the cytokines, which precludes systemic administration of optimal dose and schedule. In this sense, the secretion of cytokines by gene-modified tumor cells may more closely resemble the physiologic mode of delivery to antigen-presenting cells or lymphocytes for immune activation.

Virtually all the cytokine genes, including those for interleukin 2 (IL-2)^{11, 17, 22}, IL-4^{15, 22, 23}, IL-6²³, IL-7³², IL-12⁶, interferon γ (IFN- γ)^{14, 29, 33}, tumor necrosis factor α (TNF- α)^{2, 31}, granulocyte colony stimulating factor (G-CSF)¹⁰, and granulocyte-macrophage colony stimulating factor (GM-CSF)¹², evaluated to date have been found to be effective in at least some animal tumor models (Table 2). It appears that the magnitude of antitumor responses is dependent not only on the par-

Table 1. Involvement of cytokines in the generation of T cell responses

Step	Cytokine	
	upregulation	downregulation
Antigen recognition by Th cells	GM-CSF, IFN- γ , IL-2	IL-4, IL-10, IL-12
CTL differentiation	IFN- γ , IL-2, IL-6, IL-7, IL-12	IL-10, TGF- β
Tumor cell lysis by CTL	GM-CSF, IFN- γ , TNF- α	
MHC, ICAM-1 expression	IFN- γ , IFN- α , TNF- α	IL-10
APC differentiation, antigenic peptide processing, presentation	GM-CSF, IL-4, IL-6, IL-12	IL-10, TNF- α

Table 2. Summary of preclinical studies evaluating the most commonly tested genetically modified cytokine-secreting tumor vaccines

Cytokine	Local rejection	Systemic protection	Systemic cure	Cells mediating systemic immunity
IL-2 ^{11, 17, 22}	yes	yes	yes	CD8 ⁺ T cells
IL-4 ^{15, 22, 23}	yes	yes	yes	CD4 ⁺ and CD8 ⁺ T cells
IL-6 ²³	yes	yes	no	T cells
IL-7 ³²	yes	nt	nt	nd
IFN- γ ^{14, 29, 33}	yes	some studies yes	no	CD8 ⁺ T cells, NK cells
TNF- α ^{2, 31}	yes	no	no	nd
GM-CSF ¹²	no	yes	yes	CD4 ⁺ and CD8 ⁺ T cells, NK cells
IL-12 ⁶	yes	yes	yes	CD4 ⁺ and CD8 ⁺ T cells, NK cells
G-CSF ¹⁰	yes	nt	nt	nd

nt – not tested, nd – not determined.

ticular cytokines and the levels of their production, but also on the inherent immunologic properties of the tumor and the immunologic status of the host.

Also, most of the experiments conducted to date involved protecting naive mice from parental tumor challenge, and only a few reports have specifically addressed the question of eradicating established tumors to approximate clinical conditions in animal models. In general, vaccination therapy with cytokine gene-modified tumors alone can induce protective immunity, but it has a very limited effect against pre-existing tumor metastasis.

Costimulatory Molecules

Although the engagement of the T cell antigen receptor by the antigen/MHC complex is a primary signal for T cell activation, optimal activation requires additional costimulatory signals. In the absence of a costimulatory signal, T cells have been shown to enter a state of antigen-specific anergy or deletion¹⁹. A variety of cell surface molecules have been shown to deliver costimulatory signals, including B7, CD40L, 41BB-ligand, HSA, ICAM-1, -2, -3, VCAM-1 and LFA-3. One of these costimulatory molecules, B7-1, a natural ligand for the CD28 T cell receptor, has been assessed by a number of investigators as to its utility in the immunotherapy of cancer^{7, 19}. Genetically modified tumor cells expressing B7-1 were shown to be immunogenic in a variety of murine tumor models, such as EL4, P815^{8, 18}. On the other hand, the constitutive expression of B7-1 failed to confer immunogenicity in the tumor models MCA101, MCA102 and melanoma B16⁹. This inconsistency in results between models was argued to be due to the innate immunogenicity of the parental tumor cells. Although the transduction of B7-1 alone may not necessarily induce systemic immunity, vaccination with tumor cells expressing both B7-1 and

IFN- γ markedly enhances this activity and may define future approaches to the improvement of vaccination strategies²⁹.

Combining Vaccine Therapy with Gene-Modified Tumor Cells and Adoptive Immunotherapy

The adoptive immunotherapy of cancer has been explored extensively in several animal models and shown to be effective against established experimental tumors^{5, 24}. These findings suggested that tumor-sensitized T cells could be generated from a tumor-bearing host. It has been reported that tumor-draining lymph node (TDLN), which is well known to contain an enriched population of precursor lymphocytes of tumor-sensitized T cells, can be activated and expanded by several methods^{20, 25, 36}. These activated TDLN cells mediated a significant reduction of established tumors in a tumor specific manner^{20, 25, 26, 34-36}. The method was further evaluated in clinical trials, whereby various kinds of cancers were treated with *in vitro* activated T cells^{4, 16, 21}. Although immune effector cells generated in animal models were highly effective *in vivo*, only a limited number of cancer patients responded to the therapy and many eventually died from the dissemination of their cancer. The failure of the adoptive immunotherapy of human cancer may be due in part to the weak immunogenicity of tumor cells and the tumor-induced immunosuppression of the generation of immune effector cells.

To enhance the antitumor efficacy of adoptive immunotherapy, tumor cells genetically modified to secrete several cytokines were utilized in the vaccination phase of the immunotherapy²². Cells from lymph nodes (LN) draining these cytokine-producing weakly immunogenic tumors were activated *in vitro* and adoptively transferred to mice bearing established pulmonary metastases (Table 3). Secretion of IL-2 by tumor cells

Table 3. Adoptive immunotherapy with anti-CD3/IL-2-activated cells generated from lymph node draining cytokine-producing tumor

No. of cells transferred ^a ($\times 10^7$)	Mean number of pulmonary metastases (SEM) ^b			
	MCA205	MCA205/IL-2	MCA205/IL-4	MCA205/IL-6
1.8	42 (3) ^c	2 (1) ^{c, d, e}	23 (3) ^{c, d}	91 (6) ^c
0.6	147 (15) ^c	59 (10) ^{c, d}	103 (15) ^c	113 (17) ^c
0.2	241 (7)	141 (14) ^{c, d}	171 (14) ^c	205 (26)
0	243 (5)			

^a Inguinal LN cells obtained from mice bearing either s.c. MCA205, MCA205/IL-2, MCA205/IL-4 or MCA205/IL-6 tumors for 9 days were cultured in 2 μ g/ml of anti-CD3 mAb for 2 days and 40 U/ml of IL-2 for 3 days. These cells were given i.v. to mice with 3-day established pulmonary MCA205 metastases.

^b Lungs were harvested and metastases counted 12 days after tumor i. v. inoculation.

Significantly different from groups receiving ^c no treatment, ^d activated MCA205 TDLN cells, or ^e activated MCA205/IL-4 TDLN cells.

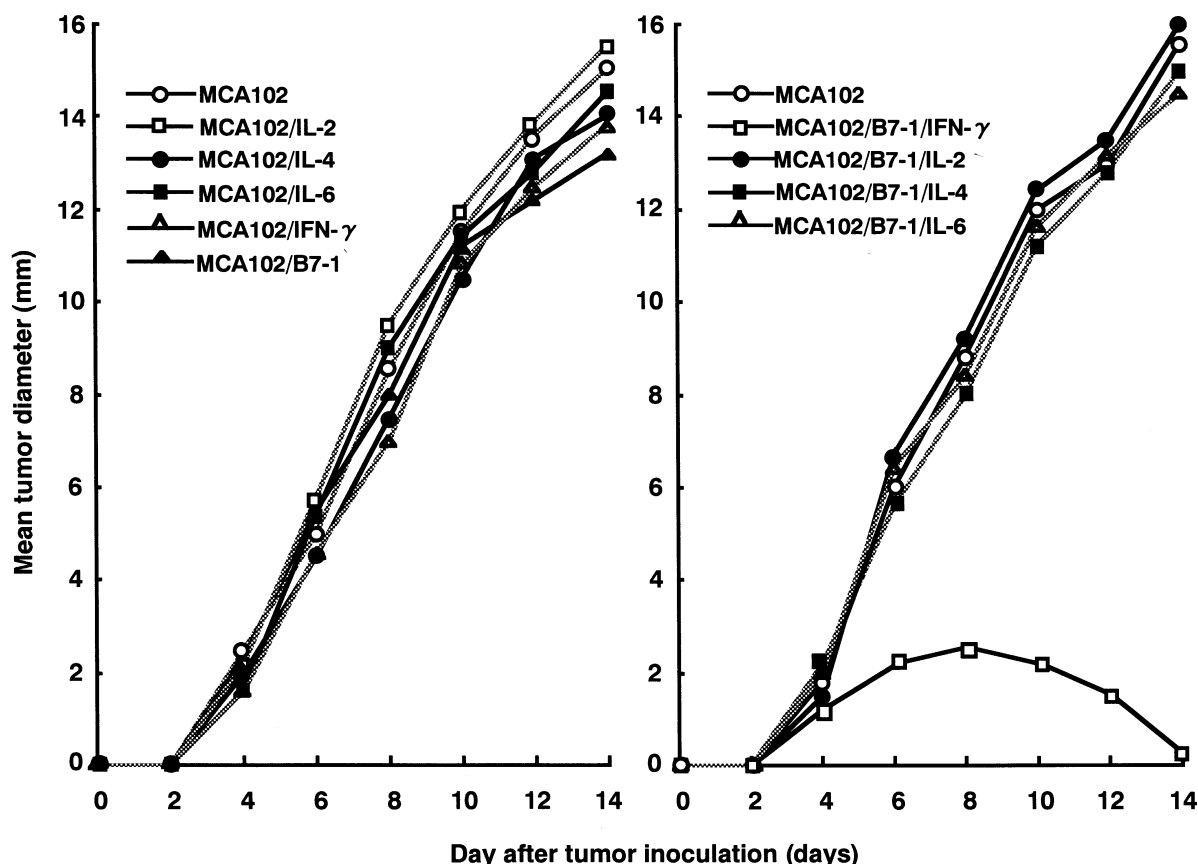


Fig. 1. *In vivo* tumor growth of gene-transduced MCA102 tumor cells in B6 mice. Cells (10^6 in 0.05 ml of HBSS) were injected s.c. into the right flank of the mice, and tumor size was measured serially. The results are expressed as mean diameter (in millimeters) of tumors from groups of five mice each

enhanced the antitumor efficacy of *in vitro* activated TDLN cells most efficiently. The mechanism of this enhancement effect was explored, revealing that tumors genetically modified to secrete IL-2 can enhance the generation of precursor lymphocytes of sensitized T cells and subsequently enhance the antitumor reactivity of adoptive immunotherapy.

The method was further explored in experimental tumors which lack apparent immunogenicity²⁹. Poorly immunogenic tumors (MCA102, LLC, B16) were transfected with cDNA for cytokines and/or B7-1. Transfection of both B7-1 and IFN- γ into MCA102 tumor cells resulted in regression of subcutaneous tumors, while the tumor transfected with other combinations of cytokine and B7-1 showed progressive growth (Fig. 1). Co-transfection of B7-1 and IFN- γ into the other poorly immunogenic tumor cells, B16 and LLC, also resulted in a regression of subcutaneous tumors (Fig. 2).

Cells from LN draining these gene-transduced tu-

mors were similarly activated *in vitro* and adoptively transferred to mice with established pulmonary metastases. Coexpression of B7-1 and IFN- γ most efficiently enhanced the antitumor effect of *in vitro* activated cells (Table 4).

These observations imply the therapeutic utility of vaccine therapy with gene-modified tumors and adoptive immunotherapy for the treatment of tumors which lack apparent immunogenicity.

Genetically Modified Dendritic Cell Vaccines

It has been demonstrated that vaccination with APC expressing tumor-related proteins and peptide improved the efficacy of active immunization. Dendritic cells (DCs) as a form of activated APC are between 100- to 1000-fold more potent than macrophages in stimulating antigen-specific T cells. The unique potency of DCs in activating T cells appears to be related to many factors.

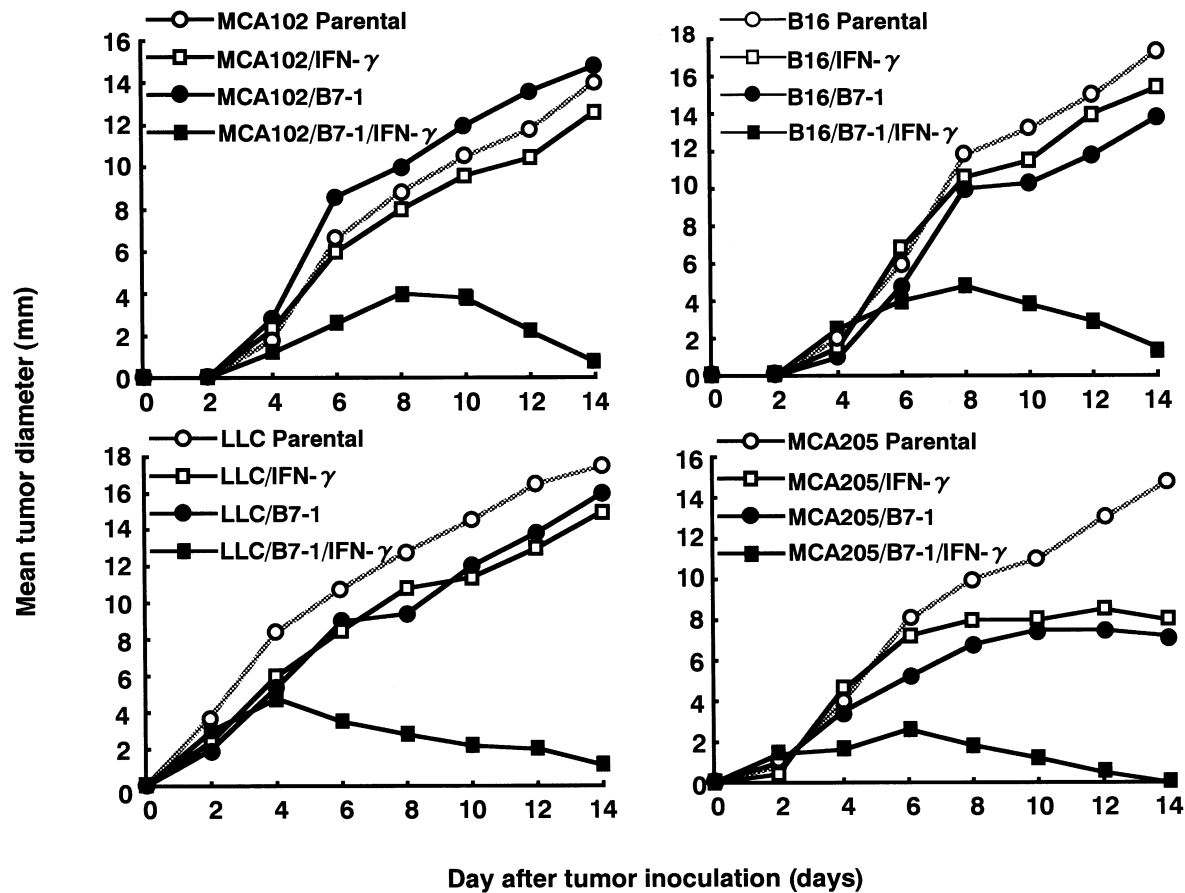


Fig. 2. *In vivo* tumor growth of B7-1- and/or IFN- γ -transfected tumor cells in B6 mice. Cells (10^6 in 0.05 ml of HBSS) were injected s.c. into the right flank of the mice, and tumor size was measured serially. The results are expressed as mean diameter (in millimeters) of tumors from groups of five mice each

Table 4. Adoptive immunotherapy with anti-CD3/IL-2-activated cells generated from LN draining B7-1- and/or IFN- γ -transfected MCA102 tumor cells

No. of cells transferred ^a ($\times 10^7$)	Mean number of pulmonary metastases (SEM) ^b			
	MCA102	MCA102/B7-1	MCA102/IFN- γ	MCA102/B7-1/IFN- γ
3	199 (41)	183 (36)	106 (22) ^{c, d, e}	14 (6) ^{c, d, e, f}
1	214 (36)	250	250 ^f	73 (15) ^{c, d, e}
0.3	250	240 (10)	216 (26)	184 (28)
0	250			

^a Inguinal LN cells obtained from mice bearing s.c. MCA102, MCA102/B7-1, MCA102/IFN- γ or MCA102/B7-1/IFN- γ tumors for 12 days were activated by the anti-CD3/IL-2 method. These cells were given i.v. to mice with 3-day established pulmonary MCA102 metastases.

^b Lungs were harvested and metastases were counted 14 days after tumor i.v. inoculation.

Significantly different from groups receiving ^c no treatment, ^d activated MCA102 tumor-draining LN cells, ^e activated MCA102/B7-1 tumor-draining LN cells or ^f activated MCA102/IFN- γ tumor-draining LN cells.

DCs express high levels of surface MHC molecules, particularly MHC class II. Thus the density of peptide/MHC ligand on the surface of a DC is much higher than that on other cells, and this is a critical parameter for T cell activation. Also, DCs express high levels of

adhesion and costimulatory molecules such as B7, ICAM-1 and LFA-1. A more recent study of other DC-specific genes, including one encoding a T cell-specific chemokine, showed their unique powers in initiating T cell responses¹.

On the basis of these fundamental studies on DC biology, a number of groups have explored *ex vivo* transduction of DCs using RNA³, replication-defective recombinant retroviral²⁸, or adeno vectors to introduce genes encoding antigens²⁷. The use of RNA for gene transfer into DCs provides a number of interesting potential advantages in that cellular RNA encoding a broad spectrum of antigens can be utilized.

Conclusions

Although previously established treatment modalities have had some success in reducing the tumor burden, a major challenge in clinical oncology remains the elimination of residual disease. There is an urgent necessity to establish an effective way to cure or at least control the progression of cancer. Progress in molecular oncology over the years has led to a better understanding of the genetic defects and immune responses in cancer. One important feature of malignancy is the genetic instability that occurs as cells progress from the benign to malignant phase. Such instability generates mutant gene products that can serve as targets of the immune system. A major priority in the field of cancer immunotherapy is the identification of mechanisms by which malignancy, particularly very weakly immunogenic cells, can evade immune detection. The studies described above provide for the possible use of gene-modification techniques in cancer therapy and may help us to understand the mechanisms of induction of host antitumor immunity as well as to establish a more potent immunotherapy for cancer.

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