Acta Medica et Biologica
Vol. 47, No.4, 131-137, 1999

Cancer Immunogene Therapy

Hirohisa YOSHIZAWA and **Fumitake GEJYO**

Department of Internal Medicine (II), Niigata University School of Medicine, Niigata, Japan

Received June 23 1999

Summary. **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; 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 the 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.

Correspondence: Hirohisa Yoshizawa MD., Department of Internal Medicine (II), Niigata University Medical School, Asahimachidori 1, Niigata 951-8510, Japan.

Introduction

Although local therapy such as surgical excision or elimination with radiotherapy remains a mainstay for the treatment of cancer, many malignancies will recur locally or, more commonly, at a distant site. Thus, the prevention of metastasis remains a major focus of clinical oncology. While chemotherapy is often the modality of choice for the treatment of an established metastasis in adjuvant settings, its use is often limited because of its toxicity and activity against many common forms of cancer. However, to improve the clinical outcome of cancer patients, immunotherapy has been considered an attractive alternative.

The most 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 are manipulated to augment immunogenicity.

Strategies to enhance the recognition of tumor cells by the immune system can be classified into 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 immuno-gene therapy for cancer takes.

Cytokine immuno-gene therapy

In 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. Those 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 systemic therapy has produced discouraging results. This could be explained in part by the toxicity of the cytokines which precludes systemic administration of optimal doses and schedules. In this sense, the secretion of cytokines by gene-modified tumor cells may more closely resemble the physiologic mode of delivery to antigen-presented cells or lymphocytes for immune activation.

Virtually, all the cytokine genes, including IL-2[1-3], IL-4[3-5], IL-6[6], IL-7[7], IL-12[8], IFN-γ[9-11], tumor necrosis factor α[12,13],granulocyte colony stimulating factor[14], and granulocyte-macrophage colony stimulating factor[15] 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 particular 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 have 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.

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-α, IFN-γ, 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	yes	yes	yes	$CD8^+$ T cells
IL-3	yes	n.t.	n.t.	n.d.
IL-4	yes	yes	yes	$CD4^+$ and $CD8^+$ T cells
IL-6	yes	yes	no	T cells
IL-7	yes	n.t.	n.t.	n.d.
IFN-γ	yes	some studies yes	no	$CD8^+$ T cells, NK cells
TNF	yes	no	no	n.d.
GM-CSF	no	yes	yes	$CD4^+$ and $CD8^+$ T cells, NK cells
IL-10	yes	yes		$CD8^+$ T cells, granulocytes
IL-12	yes	yes	yes	$CD4^+$ and $CD8^+$ T cells, NK cells
G-CSF	yes	n.t.	n.t.	n.d.

n.t., not tested; n.d., not determined.

Co-stimulatory 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 deletion[16]. A variety of cell surface molecules have been shown to deliver costimulatory signals, including B7, CD40L, 41 BB-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 immunotherapy for cancer[16,17]. Genetically modified tumor cells expressing B7-1 were shown to be immunogenic in a variety of murine tumor models such as EL4, P815[18,19]. On the other hand, the constitutive expression of B7-1 failed to confer immunogenicity in tumor models MCA 101, MCA 102 and melanoma B16[20]. This inconsistency in results between models was argued to arise from the innate immunogenicity of the parental tumor cells. Although the transduction of B7-1 alone may not necessarily induce systemic immunity, covaccination of B7-1 with IFN-γ markedly enhances this activity and may define future approaches to the improvement of vaccination strategies[10].

Combining vaccine therapy with gene-modified tumor cells and adoptive immunotherapy

The adoptive immunotherapy for cancer has been explored extensively in several animal models and shown to be effective against established experimental tumors[21,22]. These findings suggest that tumor-sensitized T cells can be generated from a tumor bearing host. It has been reported that the 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[23-25]. These activated tumor-draining LN cells mediate a significant reduction of established tumors in a tumor specific manner[23-28]. The method was further evaluated in clinical trials whereby various kinds of cancers were treated with *in vitro* activated T cells[29-31]. 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.

To enhance the antitumor efficacy of adoptive immunotherapy, tumor cells genetically modified to secrete several cytokines were utilized in the vaccination phase of the immunotherapy[3]. Cells from 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 was most efficient in enhancing the antitumor efficacy of *in vitro* activated TDLN cells. The mechanism of this enhancement effect was further 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.

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

No. of cells transferred[a]	Mean no. of pulmonary metastases (SEM)[b]			
	MCA205	MCA205/IL-2	MCA205/IL-4	MCA205/IL-6
1.8×10^7	42(3)[c]	2(1)[c,d,e]	23(3)[c,d]	91(6)[c]
0.6×10^7	147(15)[c]	59(10)[c,d]	103(15)[c]	113(17)[c]
0.2×10^7	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 μg/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.

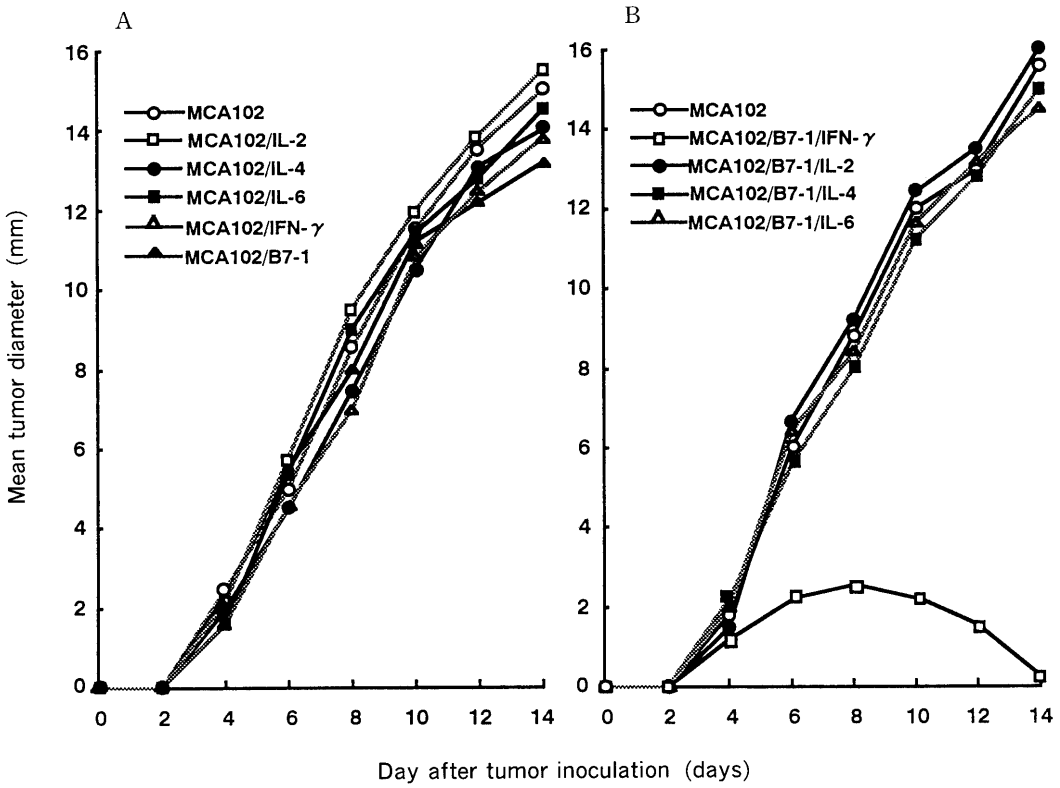

Fig. 1. *In vivo* tumor growth of gene-transduced MCA102 tumor cells in B6 mice. Cells (10^6 in 0.05 ml of HBSS) were injected s. c. into the right flank of mice, and tumor size was measured serially. The results are expressed as mean diameter (in millimeters) of tumors from groups of five mice each.

The method was further explored in experimental tumors which lacked apparent immunogenicity[10]. Poorly immunogenic tumors (MCA102, LLC, B 16) were transfected with cDNA for cytokines and/or B7-1. Transfection of both B7-1 and IFN-γ into MCA 102 tumor cells resulted in a 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 other poorly immunogenic tumor B16 and LLC cells also resulted in a regression of subcutaneous tumors (Fig. 2).

Cells from LN draining these gene-transduced tumors 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 antigen-presenting cells expressing tumor related proteins and peptides 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. DCs express high levels of surface MHC molecules, particularly MHC class II. Thus the density of the 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 responses[32].

On the basis of these fundamental studies on DC biology, a number of groups have explored the *ex*

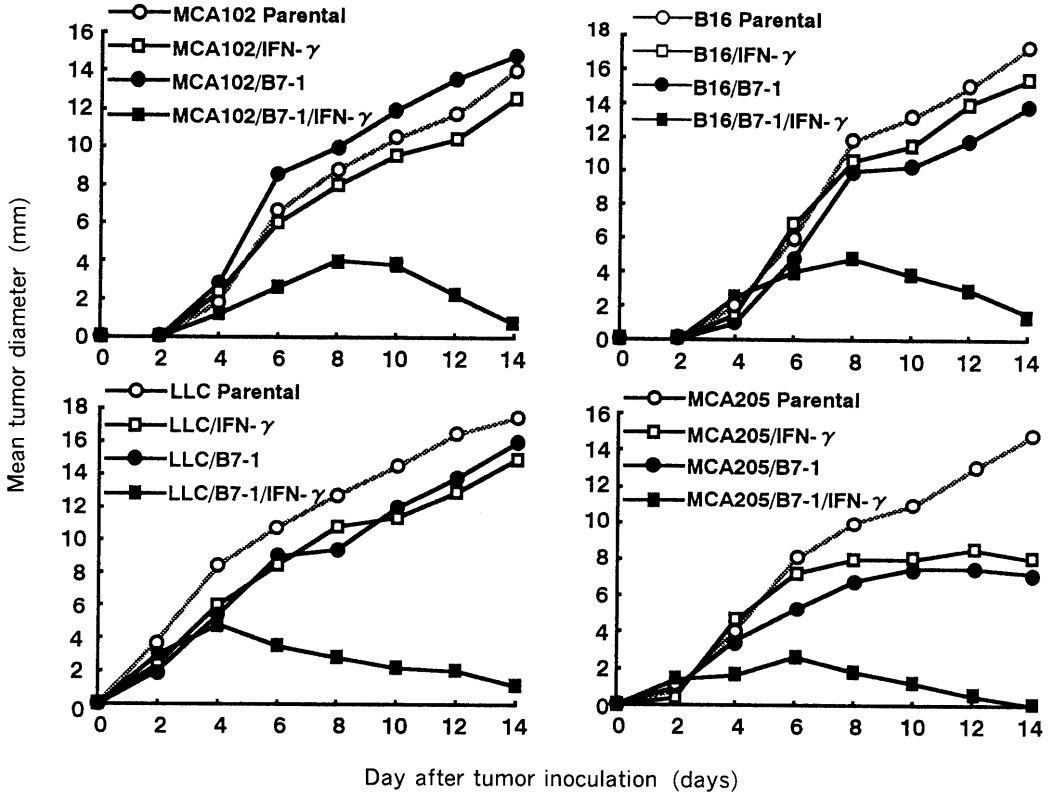

Fig. 2. *In vivo* tumor growth of B7-1- and/or IFN-γ-transfected tumor cells in B6 mice. Cells (10^6 in 0.05 ml of HBSS) were injected s. c. into the right flank of mice, and tumor size was measured serially. The results are expressed as mean diameter (in millimeters) of tumors from groups of five mice 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 no. 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.

vivo transduction of DCs using RNA[33], replication-defective recombinant retroviral[34], or adeno vectors to introduce genes encoding antigens[35]. 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 for host antitumor immunity as well as to establish a more potent immunotherapy for cancer.

References

1) Fearon ER, Pardoll DM, Itaya T, Golumbek P, Levitsky HI, Simons JW, Karasuyama H, Vogelstein B, Frost P: Interleukin-2 production by tumor cells bypasses T helper function in the generation of an antitumor response. *Cell* **60**: 397-403, 1990.
2) Karp SE, Farber A, Salo JC, Hwu P, Jaffe G, Asher AL, Shiloni E, Restifo NP, Mule JJ, Rosenberg SA: Cytokine secretion by genetically modified nonimmunogenic murine fibrosarcoma. Tumor inhibition by IL-2 but not tumor necrosis factor. *J Immunol* **150**: 896-908, 1993.
3) Ohno K, Yoshizawa H, Tsukada H, Takeda T, Yamaguchi Y, Ichikawa K, Maruyama Y, Suzuki Y, Suzuki E, Arakawa M: Adoptive immunotherapy with tumor-specific T lymphocytes generated from cytokine gene-modified tumor-primed lymph node cells. *J Immunol* **156**: 3875-3881, 1996.
4) Golumbek PT, Lazenby AJ, Levitsky HI, Jaffee LM, Karasuyama H, Baker M, Pardoll DM: Treatment of established renal cancer by tumor cells engineered to secrete interleukin-4. *Science* **254**: 713-716, 1991.
5) Tepper RI, Pattengale PK, Leder P: Murine interleukin-4 displays potent anti-tumor activity in vivo. *Cell* **57**: 503-512, 1989.
6) Porgador A, Tzehoval E, Katz A, Vadai E, Revel M, Feldman M, Eisenbach L: Interleukin 6 gene transfection into Lewis lung carcinoma tumor cells suppresses the malignant phenotype and confers immunotherapeutic competence against parental metastatic cells. *Cancer Res* **52**: 3679-3686, 1992.
7) Visse E, Siesjo P, Widegren B, Sjogren HO: Regression of intracerebral rat glioma isografts by therapeutic subcutaneous immunization with interferon-gamma, interleukin-7, or B7-1- transfected tumor cells. *Cancer Gene Ther* **6**: 37-44, 1999.
8) Chen L, Chen D, Block E, O'Donnell M, Kufe DW, Clinton SK: Eradication of murine bladder carcinoma by intratumor injection of a bicistronic adenoviral vector carrying cDNAs for the IL-12 heterodimer and its inhibition by the IL-12 p40 subunit homodimer. *J Immunol* **159**: 351-359, 1997.
9) Gansbacher B, Bannerji R, Daniels B, Zier K, Cronin K, Gilboa E: Retroviral vector-mediated gamma-interferon gene transfer into tumor cells generates potent and long lasting antitumor immunity. *Cancer Res* **50**: 7820-7825, 1990.
10) Tanaka H, Yoshizawa H, Yamaguchi Y, Ito K, Kagamu H, Suzuki E, Gejyo F, Hamada H, Arakawa M: Successful adoptive immunotherapy of murine poorly immunogenic tumor with specific effector cells generated from gene-modified tumor-primed lymph node cells. *J Immunol* **162**: 3574-3582, 1999.
11) Watanabe Y, Kuribayashi K, Miyatake S, Nishihara K, Nakayama E, Taniyama T, Sakata T: Exogenous expression of mouse interferon gamma cDNA in mouse neuroblastoma C1300 cells results in reduced tumorigenicity by augmented anti-tumor immunity. *Proc Natl Acad Sci USA* **86**: 9456-9460, 1989.
12) Asher AL, Mule JJ, Kasid A, Restifo NP, Salo JC, Reichert CM, Jaffe G, Fendly B, Kriegler M, Rosenberg SA: Murine tumor cells transduced with the gene for tumor necrosis factor-alpha. Evidence for paracrine immune effects of tumor necrosis factor against tumors. *J Immunol* **146**: 3227-3234, 1991.
13) Vanhaesebroeck B, Mareel M, Van Roy F, Grooten J, Fiers W: Expression of the tumor necrosis factor gene in tumor cells correlates with reduced tumorigenicity and reduced invasiveness in vivo. *Cancer Res* **51**: 2229-2238, 1991.
14) Colombo MP, Ferrari G, Stoppacciaro A, Parenza M, Rodolfo M, Mavilio F, Parmiani G: Granulocyte colony-stimulating factor gene transfer suppresses tumorigenicity of a murine adenocarcinoma in vivo. *J Exp Med* **173**: 889-897, 1991.

15) Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, Jackson V, Hamada H, Pardoll D, Mulligan RC: Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci USA* **90**: 3539-3543, 1993.
16) Linsley PS, Ledbetter JA: The role of the CD28 receptor during T cell responses to antigen. *Annu Rev Immunol* **11**: 191-212, 1993.
17) Chen L, Linsley PS, Hellstrom KE: Costimulation of T cells for tumor immunity. *Immunol Today* **14**: 483-486, 1993.
18) Chen L, McGowan P, Ashe S, Johnston JV, Hellstrom I, Hellstrom KE: B7-1/CD80-transduced tumor cells elicit better systemic immunity than wild-type tumor cells admixed with Corynebacterium parvum. *Cancer Res* **54**: 5420-5423, 1994.
19) La Motte RN, Sharpe AH, Bluestone JA, Mokyr MB: Host B7-1 and B7-2 costimulatory molecules contribute to the eradication of B7-1-transfected P815 tumor cells via a CD8+ T cell- dependent mechanism. *J Immunol* **162**: 4817-4823, 1999.
20) Chen L, McGowan P, Ashe S, Johnston J, Li Y, Hellstrom I, Hellstrom KE: Tumor immunogenicity determines the effect of B7 costimulation on T cell-mediated tumor immunity. *J Exp Med* **179**: 523-532, 1994.
21) Cheever MA, Greenberg PD, Fefer A: Potential for specific cancer therapy with immune T lymphocytes. *J Biol Response Mod* **3**: 113-127, 1984.
22) Rosenberg SA, Terry WD: Passive immunotherapy of cancer in animals and man. *Adv Cancer Res* **25**: 323-388, 1977.
23) Shu S, Rosenberg SA: Adoptive immunotherapy of a newly induced sarcoma: immunologic characteristics of effector cells. *J Immunol* **135**: 2895-2903, 1985.
24) Yoshizawa H, Sakai K, Chang AE, Shu SY: Activation by anti-CD3 of tumor-draining lymph node cells for specific adoptive immunotherapy. *Cell Immunol* **134**: 473-479, 1991.
25) Mitsuma S, Yoshizawa H, Ito K, Moriyama H, Wakabayashi M, Chou T, Arakawa M, Shu S: Adoptive immunotherapy mediated by anti-TCR/IL-2-activated tumour- draining lymph node cells. *Immunology* **83**: 45-51, 1994.
26) Yoshizawa H, Chang AE, Shu S: Specific adoptive immunotherapy mediated by tumor-draining lymph node cells sequentially activated with anti-CD3 and IL-2. *J Immunol* **147**: 729-737, 1991.
27) Yoshizawa H, Chang AE, Shu SY: Cellular interactions in effector cell generation and tumor regression mediated by anti-CD3/interleukin 2-activated tumor-draining lymph node cells. *Cancer Res* **52**: 1129-1136, 1992.
28) Sondak VK, Tuck MK, Shu S, Yoshizawa H, Chang AE: Enhancing effect of interleukin 1 alpha administration on antitumor effector T-cell development. *Arch Surg* **126**: 1503-1508; discussion 1508-1509, 1991.
29) Ito K, Chou T, Mitsuma S, Moriyama H, Wakabayashi M, Mizusawa A, Miyao H, Yoshizawa H, Tsuchiya T, Ebe T, Yuejing L, Arakawa M: A novel chemo-immunotherapy for advanced non-small cell lung cancer. *J Jpn Soc Cancer Ther* **29**: 36-41, 1993.
30) Miyao H, Chou T, Ito K, Moriyama H, Mitsuma S, Wakabayashi M, Yoshizawa H, Arakawa M: Treatment of advanced primary lung cancer associated with malignant pleural effusion by the combination of immunotherapy and chemotherapy. *Oncology* **51**: 87-94, 1994.
31) Chang AE, Yoshizawa H, Sakai K, Cameron MJ, Sondak VK, Shu S: Clinical observations on adoptive immunotherapy with vaccine-primed T-lymphocytes secondarily sensitized to tumor in vitro. *Cancer Res* **53**: 1043-1050, 1993.
32) Adema GJ, Hartgers F, Verstraten R, de Vries E, Marland G, Menon S, Foster J, Xu Y, Nooyen P, McClanahan T, Bacon KB, Figdor CG: A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. *Nature* **387**: 713-717, 1997.
33) Boczkowski D, Nair SK, Snyder D, Gilboa E: Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. *J Exp Med* **184**: 465-472, 1996.
34) Specht JM, Wang G, Do MT, Lam JS, Royal RE, Reeves ME, Rosenberg SA, Hwu P: Dendritic cells retrovirally transduced with a model antigen gene are therapeutically effective against established pulmonary metastases. *J Exp Med* **186**: 1213-1221, 1997.
35) Song W, Kong HL, Carpenter H, Torii H, Granstein R, Rafii S, Moore MA, Crystal RG: Dendritic cells genetically modified with an adenovirus vector encoding the cDNA for a model antigen induce protective and therapeutic antitumor immunity. *J Exp Med* **186**: 1247-1256, 1997.