Jpn. J. Cancer Res. **90**, 1000–1006, September 1999

Antitumor Effects of Interleukin-2 Gene-modified Fibroblasts in an Orthotopic Colon Cancer Model

Hiroshi Terasawa, Hiroshi Tanimura, Mikihito Nakamori, Takuya Tsunoda, Makoto Iwahashi, Masaji Tani and Hiroki Yamaue[1]

Second Department of Surgery, Wakayama Medical School, 811-1 Kimiidera, Wakayama 641-0012

We transduced the interleukin-2 (*IL-2*) gene into murine fibroblasts BALBCL7 or murine colon cancer CT26 using a retroviral vector. BALBCL7 transduced with *IL-2* gene secreted 748 pg/ml of IL-2, whereas *IL-2* gene-modified CT26 secreted 1,167 pg/ml of IL-2 (48 h incubation, 1×10^6/ml). Then, we inoculated gene-modified BALBCL7 and/or CT26 cells into BALB/c female mice, and observed the tumor growth. The tumor growth was inhibited in mice inoculated with parental CT26 plus *IL-2* gene-modified BALBCL7, compared with that in mice given parental CT26 alone ($P<0.01$). Moreover, we investigated the cytotoxic activity of spleen cells derived from mice treated with gene-modified cells, and performed phenotypic analysis of the effector cells. The killer cells derived from mice inoculated with *IL-2* gene-modified BALBCL7 plus parental CT26 showed higher cytotoxic activity than those from mice inoculated with CT26 alone. The cytotoxic activity was almost completely blocked by anti-CD8 antibody (Ab), and partially blocked by anti-asialo GM1 Ab. Next, we inoculated CT26 tumor tissue into murine cecum orthotopically, and treated the animals with gene-modified BALBCL7 plus parental CT26. The tumor size in the cecum was significantly decreased, compared with parental CT26 alone ($P<0.01$).

Key words: Gene therapy — Fibroblast — Orthotopic model — Cytokine gene

Interleukin-2 (IL-2) is an important cytokine in the generation of antitumor immunity mediated by cytotoxic T cells and natural killer cells. Several investigators have transferred *IL-2* gene into tumor cells, and in these studies, expression of *IL-2* transgenes by the tumor cells abrogated their tumorigenicity after implantation and resulted in the generation of systemic tumor cells.[1–3] However, standardized and reproducible *ex vivo* transfection and culture of tumor cells are often difficult due to heterogeneity of tumor tissues obtained from different patients and sites.[4] Therefore, some investigators have been trying to transduce the cytokine gene into fibroblasts, which can be obtained easily from human subjects and are known to proliferate stably in tissue culture.[5, 6] Moreover, it has been demonstrated that the fibroblast-mediated gene therapy resulted in immune response and tumor regression.[7–10] In this gene therapy system, repeated immunization with a mixture of irradiated tumor cells as a source of tumor-associated antigen and *IL-2* gene-transduced fibroblasts abolished visible tumors in a subset of treated animals, and generated systemic antitumor immunity and CD8(+) cytotoxic T lymphocytes (CTL).

As an animal model for human cancers, subcutaneous tumor implantation had been a standard methodology for years. Although such a model has helped us to understand the nature of human cancer and to develop therapies, major problems remain unsolved. One of them is that a tumor inoculated subcutaneously no longer behaves as it did in the human patient, because the cell lines and desegregated cells were obtained by breaking the original structure of the tumor tissue, which may lead to a change in the nature and the biological behavior of the tumor, e.g., local growth rate, or metastasis either regionally or distally.[11, 12] Therefore, we established an animal model for individual human colon cancer patients. The model involves orthotopic transplantation of histologically intact colon cancer specimens to the murine cecum, and can mimic the clinical picture, including extensive local tumor growth, metastasis to the liver, extension of the local growth to the colon and small intestine and peritoneum, and severe cachexia.[11, 12] We first tried to establish fibroblast-mediated gene therapy for the orthotopic colon cancer model and investigated its therapeutic efficacy.

MATERIALS AND METHODS

Cell lines and mice CT26 tumor cell line, an undifferentiated low-immunogenic murine colorectal adenocarcinoma cell line was established from an N-nitroso-N-methylurethane-induced transplantable tumor,[13] which was obtained from Hoffman-La Roche (Kamakura). BALBCL7 murine fibroblast cell line was purchased from the American Type Culture Collection (Rockville, MD). Both cell lines were maintained in Dulbecco's modified Eagle's medium (Nissui Pharmaceutical Co., Tokyo) sup-

[1] To whom correspondence should be addressed.
E-mail: yamaue-h@wakayama-med.ac.jp

plemented with 10% fetal bovine serum (GIBCO BRL, Grand Island, NY), 2×10^{-5} *M* 2-mercaptoethanol, 2 *M* glutamine, and antibiotics at 37°C in a 5% CO_2 air atmosphere. The animals used were 6-week-old, pathogen-free BALB/c and BALB/c *nu*/*nu* female mice (Japan SLC, Inc., Hamamatsu).

Retroviral vector constructs The retroviral vector used in this study is a high-titer vector derived from the N2 vector by replacing the 5′ long terminal repeats (LTRs) of M-MuLV with M-MSV LTR and inserting a *Bal*1/*Xma*3 fragment containing the human cytomegalovirus (HCMV) immediate early promoter 3′ to the Neo^R gene.[14] A polylinker containing *Hind*III, *Hepa*I, and *Cla*I sites was inserted 3′ to the HCMV promoter for cloning of the target gene. The 3′-end-truncated 480 bp *IL-2* gene was cleaved with *Hind*III and *Cla*I, and the gel-purified fragments were then ligated to *Hind*III/*Cla*I linearized pLNCX.[15]

Transfection of packaging cell lines The retroviral expression construct pLNCIL-2 was introduced into amphotropic PA317[16] and ecotropic ψ2[17] packaging cells by lipofection.[18] Helper virus was not present in the supernatant of the packaging cell lines. Transfectants were then selected in medium containing 1 mg/ml of the neomycin analog Geneticin (active concentration of 687 μg/mg G418, GIBCO). The supernatants of PA317 transfected with *IL-2* gene had IL-2 titers of 2,035 pg/ml (24 h incubation/1×10^6 cells) by enzyme-linked immunosorbent assay (ELISA, Intertest-2X, Genzyme, Cambridge, MA). The ψ2 secreted 1,917 pg/ml of IL-2.

Preparation of IL-2-secreting murine tumor cells and fibroblasts The *IL-2* gene and Neo^R gene that confers resistance to the aminoglycoside antibiotic, G418, were transduced to the target cells including CT26 and BALBCL7 by using the double chamber method.[15] First, the *IL-2* gene packaging cells, ψ2 and PA317, were seeded in the lower chamber for micro ping-pong reaction,[15, 19] and after 48 h, target cells were seeded in the upper chamber to allow continuous retroviral infection. Afterwards, the *IL-2* gene-transduced CT26 or BALBCL7 were selected for 7 days in growth medium containing 1 mg/ml of G418.

Assays for cytokine secretion Supernatant of *IL-2* gene-transduced cells seeded at a concentration of 1×10^6 cells/well was collected after 48 h, and assayed in duplicate for IL-2 by ELISA.[20]

Tumor transplantation BALB/c mice were inoculated subcutaneously on the back with 1×10^6 cells of *IL-2* gene-modified CT26 (CT26/IL-2), *IL-2* gene-modified BALBCL7 (BALBCL7/IL-2), nontreated CT26, nontreated BALBCL7, Neo^R gene-modified CT26 (CT26/Neo), and Neo^R gene-modified BALBCL7 (BALBCL7/Neo) in seven groups. After transplantation, the tumor sizes were measured for 40 days.

Cytotoxicity assay A 4 h ^{51}Cr-release assay was performed as previously described.[20] Briefly, target cells were labeled with 100 μCi of $Na_2{}^{51}CrO_4$, and washed three times. ^{51}Cr-labeled target cells (100 μl; 1×10^5/ml) were added in triplicate to 100 μl of effector cells. Effector-to-target ratios (E/T ratios) were varied from 50:1 to 12.5:1 in round-bottomed microtiter plates (Corning no. 25850). After 4 h of incubation at 37°C, the supernatants were counted using a γ counter. The percentage cytotoxicity was calculated as follows (all ^{51}Cr values in cpm): (experimental ^{51}Cr release−spontaneous ^{51}Cr release)/(maximum ^{51}Cr release−spontaneous ^{51}Cr release)×100.

Phenotypic analysis of effector cells activated with *IL-2* gene-modified cells To identify the effector cells involved in cell-mediated cytotoxicity, a 4 h ^{51}Cr-release assay was performed in the presence of blocking concentrations of anti-CD4 (anti-L3T4 rat IgG, Chemicon International Inc., Temecula, CA; 5 μg/1×10^5 cells), and anti-CD8 (anti-Lyt2.2 rat IgG, Chemicon International Inc.; 5 μg/1×10^5 cells) monoclonal antibodies, or anti-asialo GM1 (Wako Chemical Co., Osaka; 5 μg/1×10^5 cells) antibody.

Orthotopic transplantation procedure CT26 tumor tissues were obtained from the back of BALB/c mice. Necrotic tissues were carefully removed with scissors, and the cancerous tissues were divided into small pieces of 2 mm in diameter. Mice were anesthetized with a 2.5% solution of a mixture of 2,2,2-tribromoethanol and tri-amyl alcohol (1:1), and an incision was made through the median abdominal line and peritoneum. The cecum wall was carefully exposed and a tumor piece was sutured on the serosal surface with a 6-0 nylon thread. The cecum was then returned into the peritoneal cavity, and the abdominal wall and the skin were closed with 5-0 nylon suture. No mice rejected the orthotopically transplanted tumor tissues.

Gene therapy in the orthotopic model *IL-2* gene-modified tumor cells and fibroblasts were inactivated with mitomycin C (100 μg/ml, 45 min) and were inoculated at 1×10^6 cells/mouse with 500 μl of saline subcutaneously or intraperitoneally on days 1, 3, and 5 to BALB/c mice or BALB/c *nu*/*nu* nude mice. Twenty days after treatment with *IL-2* gene-modified cells, mice were killed and the tumors were measured.

Resected tumors were fixed in 10% buffered formalin and stained with hematoxylin and eosin for histopathological analysis.

RESULTS

IL-2 secretion of CT26/IL-2 and BALBCL7/IL-2 CT26/IL-2 and BALBCL7/IL-2 secreted 748 pg/ml, and 1,167 pg/ml of IL-2 on 48-h incubation at a concentration of 1×10^6/ml, respectively. After continuous culture for three months, or after treatment with mitomycin C, these

cells also secreted the equivalent concentrations of IL-2, whereas CT26/Neo and BALBCL7/Neo did not secrete IL-2.

Suppression of tumorigenicity by *IL-2* gene-modified cells The tumor growth was significantly suppressed in mice inoculated with CT26/IL-2, compared with other groups. CT26+BALBCL7/IL-2 also suppressed the tumorigenicity, but less effectively than CT26/IL-2. Increasing the dose of BALBCL7/IL-2 did not increase tumor suppression, though 4 of 10 mice were tumor-free, a rate equal to that in the CT26/IL-2 group (Fig. 1). When the tumor-free mice were rechallenged with 1×10^6 parental CT26 cells at day 40, all the mice rejected the parental CT26 cells.

Spleen cell-mediated cytotoxicity The cytotoxic activity in spleen cells of mice immunized with the parental CT26 was less than 10% even at the E/T ratio=50, whereas it was over 50% and 40% in spleen cells of mice immunized with CT26/IL-2 and CT26+BALBCL7/IL-2, respectively ($P<0.01$, and $P<0.05$; Table I). The cytotoxic activity induced by gene-modified cells was almost completely blocked by anti-CD8 antibody (Ab), and partially blocked by anti-asialo GM1 Ab, whereas the control Ab (rat IgG) and anti-CD4 Ab could not block these activities (Table II).

Gene therapy in the orthotopic model In the case of subcutaneous inoculation, the size of the orthotopic tumor was significantly suppressed by the vaccination of CT26/IL-2 or CT26+BALBCL7/IL-2, compared with other groups ($P<0.01$, Fig. 2). However, in the mice treated with parental CT26, the orthotopic tumors grew well, and occupied the abdominal cavity. In the nude mice, there was no therapeutic effect of the vaccination of CT26/IL-2 or CT26+BALBCL7/IL-2 in terms of tumor suppression (Table III). On the other hand, intraperitoneal vaccination never suppressed the tumor growth, even in mice treated with CT26/IL-2 and CT26+BALBCL7/IL-2 (Fig. 3).

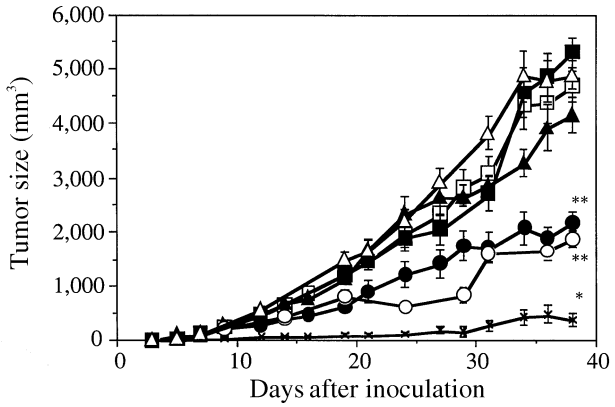

Fig. 1. Tumor growth suppression of *IL-2* gene-modified cells. *IL-2* gene-modified fibroblasts suppressed the tumor growth of parental CT26 cells. When the *IL-2* gene-modified fibroblasts were used with parental CT26 cells at 2:1 ratio, the tumor growth was suppressed compared to parental CT26 cells alone, and moreover 40% of mice became tumor-free. * $P<0.01$, compared with CT26+BALBCL7/IL-2. ** $P<0.01$, compared with CT26 alone. Ratio of tumor-free mice: □ CT26 (0/10), ■ CT26/Neo (0/10), △ CT26+BALBCL7 (0/10), ▲ CT26+BALBCL7/Neo (0/10), ● CT26+BALBCL7/IL-2 (0/10), ○ CT26+2× BALBCL7/IL-2 (4/10), × CT26/IL-2 (4/10).

Table I. Induction of CTL from Mice Inoculated with *IL-2* Gene-modified Cells (%Cx)

Immunization	E/T ratio		
	12.5	25	50
CT26	8.9±11	10±11	8.4±9.2
CT26/Neo	1.0±4.0	0.3±3.3	0.8±3.7
CT26+BALBCL7/Neo	5.3±8.8	9.5±14	5.0±7.4
CT26/IL-2	51±15	62±14	61±13[a,b)]
CT26+BALBCL7/IL-2	38±17	47±18	44±17

Mononuclear cells from the spleens of the immunized mice were incubated for 5 days with mitomycin C-treated parental CT26 cells. After incubation, the nonadherent cells were collected and the cytotoxicity toward the CT26 cells was determined in a 4-h ^{51}Cr-release assay. The data were expressed as the mean±SD.
a) $P<0.01$, compared to CT26 alone.
b) $P<0.01$, compared to CT26+BALBCL7/IL-2.

Table II. Determination of the Phenotype of Effector Cells (%Cx)

Immunization	Antibody				
	No treatment	Control Ab	Anti-CD4	Anti-CD8	Anti-asialo GM1
CT26	4.4±0.7	3.2±0.3	3.1±0.7	1.1±0.2	1.2±0.1
CT26/IL-2	32±6.2	36±7.6	41±6.2	3.9±2.8	11±3.2
CT26+BALBCL7/IL-2	21±4.6	21±5.5	23±4.6	1.5±0.1	6.3±1.7

The phenotypic analysis of effector cells was performed using various antibodies including anti-CD4 Ab, anti-CD8 Ab, and anti-asialo GM1 Ab. The control Ab used was rat IgG. The data were expressed as the mean±SD.

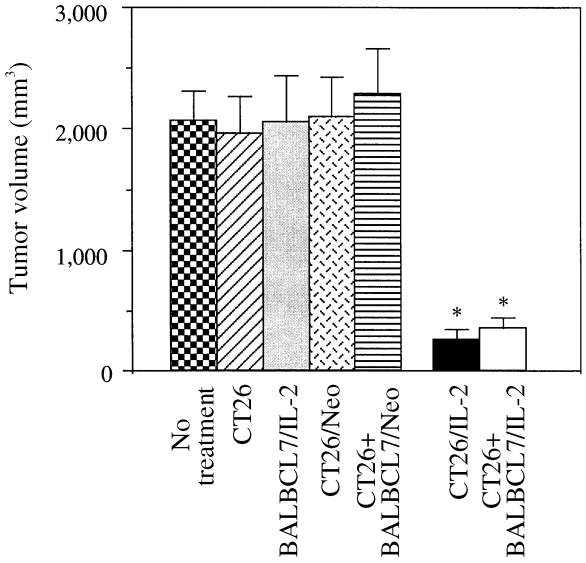

Fig. 2. Antitumor effects of subcutaneous injection with *IL-2* gene-modified cells in orthotopic models. The tumor growth of the mice treated with CT26 IL-2 or CT26+CL7 IL-2 was significantly suppressed compared with other groups. Each group: n=10. * P<0.01, compared with other groups.

Histopathological analysis of orthotopic tumor The orthotopic tumor was remarkably diminished by vaccination with *IL-2* gene-modified fibroblasts, and histopathological analysis was performed to investigate whether immunocompetent cells are accumulated in the tumor tissues. Fig. 4A shows the orthotopic tumor treated with parental CT26; the viable undifferentiated colon adenocarcinoma cells aggressively proliferated, whereas in contrast, numerous lymphocytic cells infiltrated into tumor tissues in mice treated with *IL-2* gene-modified fibroblasts (Fig. 4B).

Table III. Antitumor Effects of Subcutaneous Inoculation with *IL-2* Gene-modified Cells in Nude Mice

Treatment	Tumor volume (mm^3)
CT26	860±160
CT26/IL-2	760±180
CT26+BALBCL7/IL-2	700±57

There was no therapeutic effect of gene therapy, even in mice treated subcutaneously with *IL-2* gene-modified cells. The data were expressed as the mean±SD. Each group: n=5.

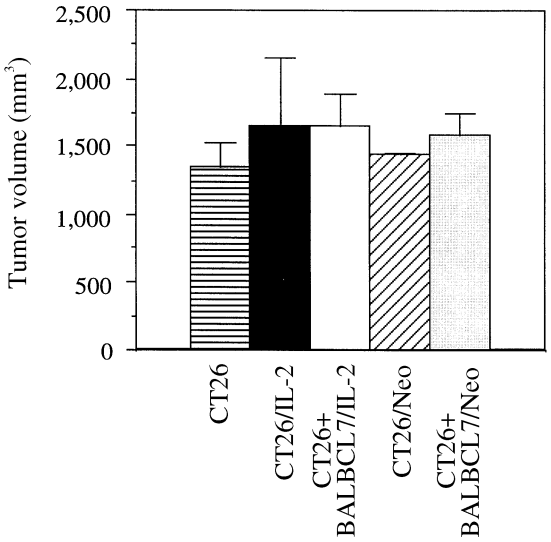

Fig. 3. Antitumor effects of intraperitoneal injection with *IL-2* gene-modified cells in orthotopic models. Intraperitoneal vaccination did not suppress tumor growth even in mice treated with CT26/IL-2 or CT26+BALBCL7/IL-2. Each group: n=10.

DISCUSSION

It has been demonstrated that *IL-2* gene therapy can induce potent antitumor immunity capable of rejecting a challenge with the parental tumor cells.[1–3] These protocols used immunotherapy approaches that consist of inoculations with tumor cells that have been genetically modified to secrete IL-2. A key requirement of these approaches is the presentation of tumor-associated antigens within the microenvironment of cytokine secretion. However, autologous tumor cells derived from cancer patients can sometimes be difficult to culture. Therefore, *IL-2* gene therapy has been attempted by vaccination with *IL-2* gene-modified fibroblasts mixed with tumor cells.[10] In these studies, a fibroblast-mediated strategy employing *IL-2* gene-modified fibroblasts mixed with irradiated tumor cells effectively induced strong antitumor immunity, like the tumor cell-mediated strategy, and this antitumor lytic activity was inhibited by anti-CD8 Ab. In our study, the cytotoxic activity induced by gene-modified cells was almost completely blocked by anti-CD8 Ab, and partially blocked by anti-asialo GM1 Ab. This finding can be explained by previous studies which indicated that low doses of IL-2 activate CD8(+) CTL, and high doses of IL-2 activate natural killer and lymphokine-activated killer cells.[10] Furthermore, our data suggest that the antitumor lytic activities parallel the growth suppression of subcutaneously inoculated tumors.

In the present study, we used the orthotopic model for gene therapy research. Different from the subcutaneous tumor model, the orthotopic model shows local growth, liver metastasis, colonic obstruction, and abdominal carcinomatosis similar to those seen in real colon cancer

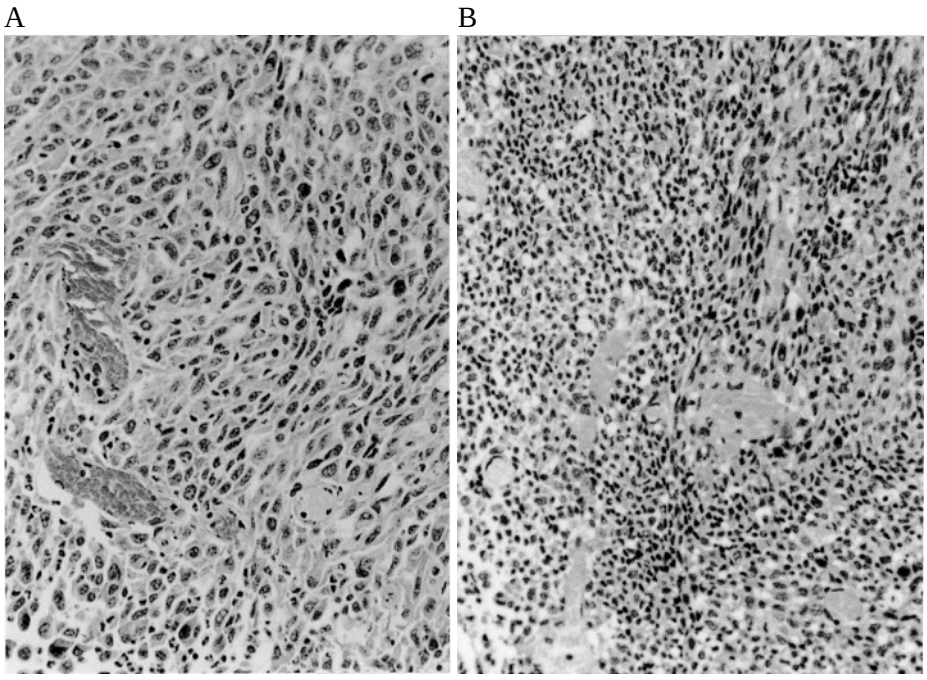

Fig. 4. Histopathological analysis of orthotopically transplanted CT26 tumor 20 days after s.c. treatments (H&E, ×200). In the tumor tissue from untreated mice, there are no infiltrating lymphocytes (a) but in the tumor tissue of treated mice, there are many infiltrating lymphocytes (b).

patients.[11, 12] Thus, this is considered a suitable model to study the clinical effects of gene therapy.

First, we established the orthotopic models on day 0, and vaccinated the animals with *IL-2* gene-modified cells once on day 1. There was no therapeutic efficacy in terms of tumor suppression, compared to the control group on day 20 (data not shown). Second, to obtain a stronger immune response, the gene-modified cells were vaccinated three times on days 1, 3, and 5. In this case, significant suppression of local tumor growth was recognized, with massive lymphocyte infiltration into the tumor tissues. We also established orthotopic models in nude mice, and vaccinated them similarly. However, no tumor suppression was seen even with three vaccinations. These data clearly indicate that the tumor suppression was mediated via T cell immunity.

Next, to determine the efficacy of intraperitoneal vaccinations, we inoculated the *IL-2* gene-modified cells in the orthotopic models intraperitoneally on days 1, 3, and 5. However, there was no suppression of tumor growth. CD8 (+) lymphocytes were activated via a class I pathway involving mainly dendritic cells in the skin by the subcutaneous vaccinations, whereas the CD4(+) lymphocytes were activated via a class II pathway involving mainly macrophages inside the peritoneum by the peritoneal vaccinations.[21, 22] In the present study, the main effector cells lysing the tumor cells were considered to be CD8(+) lymphocytes, in accordance with the difference of therapeutic efficacy between the subcutaneous and intraperitoneal vaccinations.

We also investigated the survival of the treated mice. However, the survival was not prolonged even in mice in which the local tumors were completely suppressed by the treatment with *IL-2* gene-modified cells (data not shown). This may be because severe cachexia and multiple liver metastases were present in all the orthotopic models. Therefore, the orthotopic model is considered to be more malignant than the subcutaneous tumor model, and new strategies will be necessary to achieve cure in this model. One possibility is combination therapy using a suicide gene and *IL-2* gene. The combination of genes has been shown to be more effective than either gene alone.[23] Another possibility is the blocking of IL-6 by administration of anti-IL-6 monoclonal antibody, because there are high levels of IL-6 in the sera, and cachexia depends on the IL-6 level in CT26 tumor-bearing mice.[24] On the other hand, it has been reported that there is an optimal dose of IL-2 to activate antitumor immune responses in *IL-2* gene therapy.[25] Thus, it is necessary to establish clones which can secrete the optimal dose of IL-2 for therapeutic effi-

cacy in the orthotopic tumor models, which closely resemble the condition of human patients. In this study, a fibroblast-mediated gene therapy system did not show any superiority over tumor cell-mediated gene therapy. However, it could induce strong antitumor immunities, which may be useful in the clinical situation, because fibroblasts are much easier to culture *in vitro* for gene modification and to use repeatedly for vaccinations, compared with freshly isolated tumor cells.

(Received March 17, 1999/Revised June 7, 1999/Accepted July 1, 1999)

REFERENCES

1) Gansbacher, B., Zier, K., Daniels, B., Cronin, K., Bannerji, R. and Gilboa, E. Interleukin 2 gene transfer into tumor cells abrogates tumorigenicity and induces protective immunity. *J. Exp. Med.*, **172**, 1217–1224 (1990).
2) Fearon, E. R., Pardoll, D. M., Itaya, T., Golumbek, P., Levitsky, H. I., Simons, J. W., Karasuyama, H., Vogelstein, B. and Frost, P. Interleukin-2 production by tumor cells bypasses T helper function in the generation of an antitumor response. *Cell*, **60**, 397–403 (1990).
3) Conne, J., Bannerji, R., Saito, S., Heston, W., Fair, W. and Gilboa, E. Regression of bladder tumors in mice treated with interleukin-2 gene-modified tumor cells. *J. Exp. Med.*, **177**, 1127–1134 (1993).
4) Yannelli, J. R., Hyatt, C., Johnson, S., Hwu, P. and Rosenberg, S. A. Characterization of human tumor cell lines transduced with the cDNA encoding either TNF-α or IL-2. *J. Immunol. Methods*, **161**, 77–90 (1993).
5) Veelken, H., Jesuiter, H., Mackensen, A., Kulmburg, P., Schultze, J., Rosenthal, F., Mertelsmann, R. and Lindemann, A. Primary fibroblasts from human adults as target cells for *ex vivo* transfection and gene therapy. *Human Gene Ther.*, **5**, 1203–1210 (1994).
6) Elder, E. M., Lotze, M. T. and Whiteside, T. L. Successful culture and selection of cytokine gene-modified human dermal fibroblasts for the biologic therapy of patients with cancer. *Human Gene Ther.*, **7**, 479–487 (1996).
7) Kim, T. S. and Cohen, E. P. Immunization of mice with allogenic fibroblasts genetically modified for interleukin-2-secretion and expression of melanoma-associated antigens stimulate predetermined classes of anti-melanoma effector cells. *J. Immunother.*, **16**, 24–35 (1994).
8) Tahara, H., Zeh, H. J., III, Storkus, W. J., Pappo, I., Watkins, S. C., Gubler, U., Gubler, S. F., Robbins, P. D. and Lotze, M. T. Fibroblasts genetically engineered to secrete interleukin-12 can suppress tumor growth and induce antitumor immunity to a murine melanoma *in vivo*. *Cancer Res.*, **54**, 182–189 (1994).
9) Glick, R. P., Lichtor, T., Kim, T. S., Ilangovan, S. and Cohen, E. P. Fibroblasts genetically engineered to secrete cytokines suppress tumor growth and induce antitumor immunity to a murine glioma *in vivo*. *Neurosurgery*, **36**, 548–555 (1995).
10) Shawler, D. L., Dorigo, O., Gjerset, R. A., Royston, I., Sobol, R. E. and Fakhrai, H. Comparison of gene therapy with interleukin-2 gene modified fibroblasts and tumor cells in the murine CT26 model of colorectal carcinoma. *J. Immunother.*, **17**, 201–208 (1995).
11) Fu, X., Besterman, J. M., Monosov, A. and Hoffman, R. M. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. *Proc. Natl. Acad. Sci. USA*, **88**, 9345–9349 (1991).
12) Fu, X., Guadagni, F. and Hoffman, R. M. A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically with histologically intact patient specimens. *Proc. Natl. Acad. Sci. USA*, **89**, 5645–5649 (1992).
13) Brattain, M. G., Strobel-Stevens, J., Fine, D., Webb, M. and Sarrif, A. M. Established mouse colonic carcinoma cell lines with different metastatic properties. *Cancer Res.*, **40**, 2142–2146 (1980).
14) Miller, A. D. and Rosman, G. J. Improved retroviral vectors for gene transfer and expression. *Biotechniques*, **7**, 980–990 (1989).
15) Yamaue, H., Kasshmiri, S. V. S., Fillipi, R. D., Nieroda, C., Yannelli, J. R., Tsang, K. Y. and Schlom, J. Enhanced interleukin-2 production in human tumor-infiltrating lymphocytes engineered by 3′-truncated interleukin-2 gene. *J. Immunother.*, **16**, 262–274 (1994).
16) Mann, R., Mulligan, R. C. and Baltimore, D. Construction of a retrovirus packaging mutant and its use to produce helper-free defective retrovirus. *Cell*, **33**, 153–159 (1983).
17) Miller, A. D. and Buttimore, C. Redesign of retrovirus packaging cell lines to avoid recombination leading to helper virus production. *Mol. Cell. Biol.*, **6**, 2895–2902 (1986).
18) Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M. and Danielsen, M. Lipofection: a highly efficient, lipid-mediated DNA transfection procedure. *Proc. Natl. Acad. Sci. USA*, **84**, 7413–7417 (1987).
19) Kozak, S. L. and Kabat, D. Ping-pong amplification of a retroviral vector achieves high-level gene expression. *J. Virol.*, **64**, 3500–3508 (1990).
20) Yamaue, H., Tanimura, H., Iwahashi, M., Tani, M., Tsunoda, T., Tabuse, K., Kuribayashi, K. and Saito, K. Role of IL-2 and IFN-γ in induction of activated natural killer cells from mice primed *in vivo* and subsequently challenged *in vitro* with the streptococcal preparation of OK432. *Cancer Immunol. Immunother.*, **29**, 79–86 (1989).
21) Ragg, S. J., Dandie, G. W., Woods, G. M. and Muller, H. K. Dendritic cells migrating from carcinogen-treated skin have reduced antigen-presenting function. *Adv. Exp. Med. Biol.*, **2**, 237–241 (1995).

22) Ribas, A., Butterfield, L. H., McBride, W. H., Jilani, S. M., Bui, L. A., Vollmer, C. M., Lau, R., Dissette, V. B., Hu, B., Chen, A. Y., Glaspy, J. A. and Economou, J. S. Genetic immunization for the melanoma antigen MART/Melan-A using recombinant adenovirus-transduced murine dendritic cells. *Cancer Res.*, **57**, 2865–2869 (1997).
23) Chen, S.-H., Kosai, K., Xu, B., Pham-Nguyen, K., Contant, C., Finegold, M. J. and Woo, S. L. C. Combination suicide and cytokine gene therapy for hepatic metastasis of colon carcinoma: sustained antitumor immunity prolongs animal survival. *Cancer Res.*, **56**, 3758–3762 (1996).
24) Fujimoto-Ouchi, K., Tamura, S., Mori, K., Tanaka, Y. and Ishitsuka, H. Establishment and characterization of cachexia-inducing and noninducing clones of murine colon 26 carcinoma. *Int. J. Cancer*, **61**, 522–528 (1995).
25) Fakhrai, H., Shawler, D. L., Gjerset, R., Naviaux, R. K., Koziol, J., Royston, I. and Sobol, R. E. Cytokine gene therapy with interleukin-2-transduced fibroblasts: effects of IL-2 dose on anti-tumor immunity. *Human Gene Ther.*, **6**, 591–601 (1995).