Jpn. J. Cancer Res. 85, 315–324, March 1994

Augmented Systemic Immunity in Mice Implanted with Tumor Necrosis Factor-α Gene-transduced Meth-A Cells

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Syngeneic BALB/c mice bearing methylcholanthrene-induced fibrosarcoma (Meth-A) cells transduced with a tumor necrosis factor (TNF) gene showed a longer life span and tumor regression as compared with mice carrying TNF-non-producing Meth-A cells. To elucidate the mechanism of the reduced tumorigenicity of TNF-producing Meth-A, we compared systemic immune responses between mice bearing high TNF producer (C5) and unmodified Meth-A cells (M0). The results indicated that the cytotoxic activity of lymphokine-activated killer cells (LAK) induced from spleen cells of mice bearing C5 was higher against both M0 and C5 than that of LAK from mice bearing M0. Also, C5 was more sensitive to LAK induced from spleen cells of C5- and M0- bearing mice than M0. We also found that cytotoxic T lymphocyte from spleen cells of mice transplanted with C5 was more cytotoxic to M0 than that from mice with M0. In addition, the population of Lyt2 (CD8)-positive T cells was higher in freshly isolated spleen cells of mice transplanted with C5 than from mice with M0. Finally, we observed a higher expression of MHC class 1 antigen on C5 than on M0. These observations suggest that the augmented host systemic immunity in mice carrying TNF gene-modified Meth-A cells is one of the mechanisms of the reduced tumorigenicity of C5 and that the increased systemic immunity can be ascribed to the increased immunogenicity of the tumor cells. Thus, the use of TNF gene-modified tumor cells as vaccines appears to be promising for therapeutic and/or prophylactic application.

Key words: Gene therapy — TNF gene — Retrovirus vector — Reduced tumorigenicity — Augmented systemic immunity

Tumor necrosis factor (TNF)-α is a cytokine originally recognized as a serum protein inducing hemorrhagic necrosis of some transplanted tumors *in vivo*.[1,2] TNF has multiple biological effects. It is a potent cytotoxic agent to some tumor cells, operating in a receptor-mediated manner.[3–6] It shows many immunomodulatory activities, such as enhancement of the HLA antigen expression on tumor cells[7] and of the interleukin-2 (IL-2) receptor expression on lymphocytes,[8] reinforcement of induction of other cytokines,[9–13] enhancement of monocyte, natural killer cell (NK) and neutrophil cytotoxic activities[14–18] and augmentation of cytotoxic T lymphocyte (CTL) and lymphokine-activated killer cell (LAK) development.[19–22]

Several clinical trials of TNF for cancer therapy have been performed, with mixed results[23,24]; systemic administration of therapeutically effective concentrations tended to give rise to intolerable side effects.[25–27]

Recently adoptive immunotherapy after transfection of tumor-infiltrating lymphocytes with the TNF gene has been attempted[28,29] in the hope of overcoming the difficulties associated with the systemic administration of TNF. Several other investigators have described reduced tumorigenicity after transduction of the TNF gene into tumor cells in animal models and postulated the involvement of immune response in this effect on the basis of the fact that the regression of TNF-producing tumors was abrogated by *in vivo* depletion of T cell subsets or inflammatory cells such as macrophages using respective antibodies.[30,31] However, no direct evidence of enhanced host "systemic" immunity has yet been presented.

In this study we first confirmed previous observations of the reduced tumorigenicity of TNF gene-transduced tumor cells *in vivo*, and then we demonstrated elevated cytotoxicity of splenic CTL and LAK from tumor-bearing mice. A mechanism of the decreased tumorigenicity *in vivo* is postulated.

MATERIALS AND METHODS

Animals Female BALB/c mice were purchased from Charles River Japan, Inc. (Tokyo) and fed in a specific pathogen-free environment. Six- to ten-week-old mice were used in the experiment.

Tumor cells Meth-A, (M0, 3-methylcholanthrene-induced fibrosarcoma cell line, syngeneic to BALB/c mice) was grown in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum

(FCS, Flow Laboratories, McLean, VA), 100 U/ml penicillin and 100 mg/ml streptomycin, and maintained at 37°C in a humidified atmosphere containing 5% CO_2. Viability of the tumor cells used in this experiment was consistently more than 95% when evaluated by the trypan blue exclusion method.

Cytokines Recombinant human TNF-α (2.37×10^6 units/mg protein), recombinant human interleukin 2 (IL-2, 4.2×10^4 units/mg protein), recombinant human interleukin 6 (IL-6, 5.0×10^6 units/mg protein), and recombinant human interferon γ (IFN-γ, 1.0×10^7 units/mg protein) were kindly provided by Asahi Chemical Industries Co., Ltd., Takeda Pharmaceutical Co., Ltd., Ajinomoto Co., Ltd., and The Green Cross Corporation, respectively.

Vectors A human TNF-expressing vector (pLJ-TNF) was constructed by inserting a human TNF cDNA derived from pcDV-TNF vector[32] into the unique *Bam*HI site of a retroviral vector pLJ[33] (a kind gift from Dr. G. Wu, University of Connecticut School of Medicine, Farmington, CT).

Briefly, pcDV-TNF vector was digested by *Bam*HI (Takara Shuzo Co., Ltd., Kyoto) and a 0.9-kb fragment coding for human TNF was isolated from the agarose gel by electroelution. After purification through phenol/chloroform extraction and ethanol precipitation, the fragment was inserted into the unique *Bam*HI site of pLJ vector by a standard ligation method. *Escherichia coli* HB101 (Takara Shuzo) was transformed with this ligation mixture and the resultant pLJ-TNF vector from *E. coli* was characterized by restriction enzyme digestion.

Another retroviral vector, pLNL6[34] (a kind gift from Dr. A. D. Miller, Fred Hutchinson Cancer Research Center, Seattle, WA), which contains the neomycin phosphotransferase gene (neo^R), was used for control experiments.

Virus-producing cell lines The ecotropic packaging cell line ΨCRE and amphotropic packaging cell line ΨCRIP,[35] which were kindly provided by Dr. R. C. Mulligan, Whitehead Inst., Cambridge, MA, were grown in DMEM (GIBCO) supplemented with 10% heat-inactivated calf serum (CS, Flow Laboratories), 100 U/ml penicillin and 100 mg/ml streptomycin. ΨCRE was transfected with either pLJ-TNF or pLNL6 by the calcium phosphate coprecipitation method.[36] Two days after the transfection, the cultured supernatant of the transfectants was collected and used to transduce the amphotropic packaging cell line, ΨCRIP, in the presence of 8 μg of polybrene per ml (Sigma Chemical Co., St. Louis, MO). The transduced cells were selected with G418 (Sigma) at 450 μg/ml for 14 days and virus-producing clones, A103 (pLJ-TNF introduced) and ANeo (pLNL6 introduced) were isolated. Clones A103 and ANeo produced infectious retroviruses at titers of about 10^5 and 10^6 G418-resistant NIH3T3 colony-forming units/ml, respectively, according to a titer assay.[37]

TNF gene transfer into Meth-A cells and selection of transduced clones Meth-A cells were incubated with a viral solution of A103 or ANeo in the presence of 8 μg/ml of polybrene for 2 h at 37°C. Then, the infected cells were washed and resuspended in RPMI 1640 medium supplemented with 10% FCS. Neomycin-resistant cells were selected with G418 (400 μg/ml) for 14 days from the 3rd day after infection. The selected transformants were cloned by a limiting dilution method. TNF production of each clone was determined by ELISA as described below.

Assay of TNF The TNF concentrations in the cell culture supernatants or in the fresh preparations of tumor extract were determined by immunoquantitative ELISA[38] (Asahi Chemical). The sensitivity limit of the ELISA kit was 0.2 U/ml.

Implantation of tumor cells To examine the tumorigenicity of transformants, 2×10^6 cells in 0.2 ml of RPMI 1640 medium were implanted subcutaneously into the right flank of BALB/c mice and the mice were monitored for tumor progression. To examine the vaccination effect of C5, 2×10^6 M0 cells were rechallenged at the left flank two weeks after complete regression of previously inoculated C5. The tumor diameter was measured in two orthogonal dimensions with a caliper and the tumor volume was calculated by using the following formula[39]:

$$V\ (mm^3) = 0.4 \times a \times b^2$$

where a is the larger and b is the smaller diameter.

TNF content in implanted tumors Fourteen days after implantation of TNF gene-transduced Meth-A cells into syngeneic BALB/c mice, tumors were removed surgically and each tumor tissue was minced in a homogenizer in PBS (pH 7.2). After centrifugation at 4000 rpm for 10 min, the supernatant was assayed for TNF concentration by the ELISA method.

Mixed lymphocyte-tumor cell culture (MLTC) to obtain CTL Tumor cells (2×10^6) were implanted into syngeneic mice as described above. Three weeks after implantation, the spleen was removed aseptically, minced into small pieces with scissors, and passed gently through 40 mesh and 100 mesh nylon screens. The cells were centrifuged at 400g for 5 min and the pellet was resuspended in a buffered ammonium chloride solution to lyse red blood cells. Then, the cells were washed twice and resuspended in a conditioned medium (CM) consisting of RPMI 1640 supplemented with 10% FCS, 100 U/ml penicillin, 100 mg/ml streptomycin, 1 mM sodium pyruvate (GIBCO), 0.1 mM non-essential MEM amino acid (GIBCO) and 5×10^{-5} M 2-mercaptoethanol.

Splenocytes (2×10^7) were co-cultured with 4×10^5 irradiated (2,000 rads) Meth-A (M0) cells in 2 ml of CM

in a 12-well plate for 6 days at 37°C under 5% CO_2 with 100 BRM U/ml of IL-2. For the first 4 days, 5 U/ml of IFN-γ was added to the medium and for the last two days, 10 U/ml of IL-6 was added.

Induction of LAK Spleen cells were obtained as described above. The cells were resuspended in CM in the presence of recombinant human IL-2 (800 BRM U/ml) at 37°C for 3–5 days.

^{51}Cr-release assay Cytotoxic activity of induced CTL or LAK was determined by a 12-h ^{51}Cr-release assay.[40] Briefly, 5×10^3 radiolabeled target cells were incubated with various ratios of CTLs or LAKs at a final volume of 0.2 ml in a 96-well round-bottomed culture plate. After 12 h of incubation, the supernatant was obtained and the radioactivity was determined in a γ counter.

Percentage cytotoxicity was calculated by applying the following formula:

% cytotoxicity = (experimental count − spontaneous count)/(total count − spontaneous count) × 100 (%).

Analysis of surface antigen by flow cytometry TNF gene-transduced clone and parental Meth-A cells were stained with a mouse monoclonal antibody (mAb) against allogeneic mouse MHC class-1 antigen (Meiji Nyugyo Co., Ltd., Tokyo), followed by a goat anti-mouse FITC-conjugated Ab (Kirkegaard & Perry Laboratories Inc., MD) and rat mAbs against mouse ICAM-1 (YN1/1,7,4),[41] VCAM-1 (M/K-1),[42] LFA-1 (FD441.8), VLA-4 (PS/2), Mel-14 (Mel-14) and CD 44 (KM201),[43] followed by a mouse anti-rat FITC-conjugated mAb (MAR18.5)[43] (these Abs were kindly provided by Dr. T. Uede, Section of Immunopathogenesis, Institute of Immunological Science, Hokkaido University, Sapporo).

Freshly isolated splenocytes from mice implanted with TNF gene-transduced clones or parental Meth-A cells and those from normal mice were stained with FITC-labeled anti-Thy1 (Becton Dickinson), anti-Lyt1 (Becton Dickinson), anti-Lyt2 (Becton Dickinson), and PE-labeled anti-L3T4 (Becton Dickinson).

These preparations were analyzed by flow cytometry using a Cytron^R (Ortho Diagnostic Systems Inc., Raritan, NJ).

RESULTS

TNF production in TNF gene-transduced tumor cells Twenty-three clones of the TNF gene-transduced Meth-A fibrosarcoma cells were obtained by limiting dilution after G418 selection. Three of these (E10, B1, C5) were studied in the present investigation. By ELISA, E10, B1 and C5 were found to produce TNF at concentrations of <0.2 U/ml, 1.5 U/ml and 17.8 U/ml per 10^5 cells in 24 h, respectively (Table I). Neomycin-resistance gene (pLNL 6)-transduced Meth-A cells (ML) and parental Meth-A cells (M0) did not secrete any detectable TNF in their culture medium.

Table I. TNF Production of TNF Gene-transduced Meth-A Clones

Clone	TNF production (U/ml)
M0 (parent Meth-A)	<0.2 [a]
ML (neo^R gene-transduced Meth-A)	<0.2
E10 (low TNF producer)	<0.2
B1 (moderate TNF producer)	1.5
C5 (high TNF producer)	17.8

a) The minimum sensitivity of ELISA was 0.2 U/ml.

The presence of the proviral sequences in transduced tumor cells was confirmed by Southern blot analysis with probes for the TNF cDNA and the neo^R gene. The expected 0.9-kb *Bam*HI DNA fragment containing exogenous TNF gene and 2.8-kb *Hin*dIII and *Xba*I DNA fragment containing neo^R gene was detected in every transduced clone (data not shown); thus the integrated genomes were presumed to be intact.

***In vivo* tumorigenicity of TNF gene-transduced clones** The tumor development of TNF gene-transduced clones implanted in syngeneic BALB/c mice is shown in Fig. 1. There were no significant differences in growth of tumors of low-producer (E10), neo^R gene-transduced clone (ML) and parental cells (M0). However, tumors of intermediate-producer (B1) and high-producer (C5) cells showed impaired growth with a significant difference at 20 days after inoculation as compared to those of E10, ML and M0. In particular, with C5, complete regression occurred 39 days after inoculation in every mouse, despite the initial growth.

Production of TNF by tumors A fresh preparation of a tumor extract at day 14 of transplantation was assayed for its TNF content. A significantly higher concentration of TNF was detected in the preparation of the C5 tumor as compared to that of the B1 tumor. In the extracts of M0, ML and E10 tumors, TNF was not detectable (Fig. 2). These observations in growing tumors were compatible with the *in vitro* results obtained with the corresponding transformants.

Survival of mice implanted with transformants Fig. 3 shows the survival rates of mice implanted with various transformants. The 50% survival rate for mice inoculated with parental cells was 36 days, while the survival rates of mice with TNF-producing cell clones B1 and C5 were significantly extended; in particular, the mice given C5 showed a 100% survival rate during the observation period (50 days).

Regression of rechallenged M0 tumor Cells of M0 (2×10^6) were inoculated into the left flank of the mice which

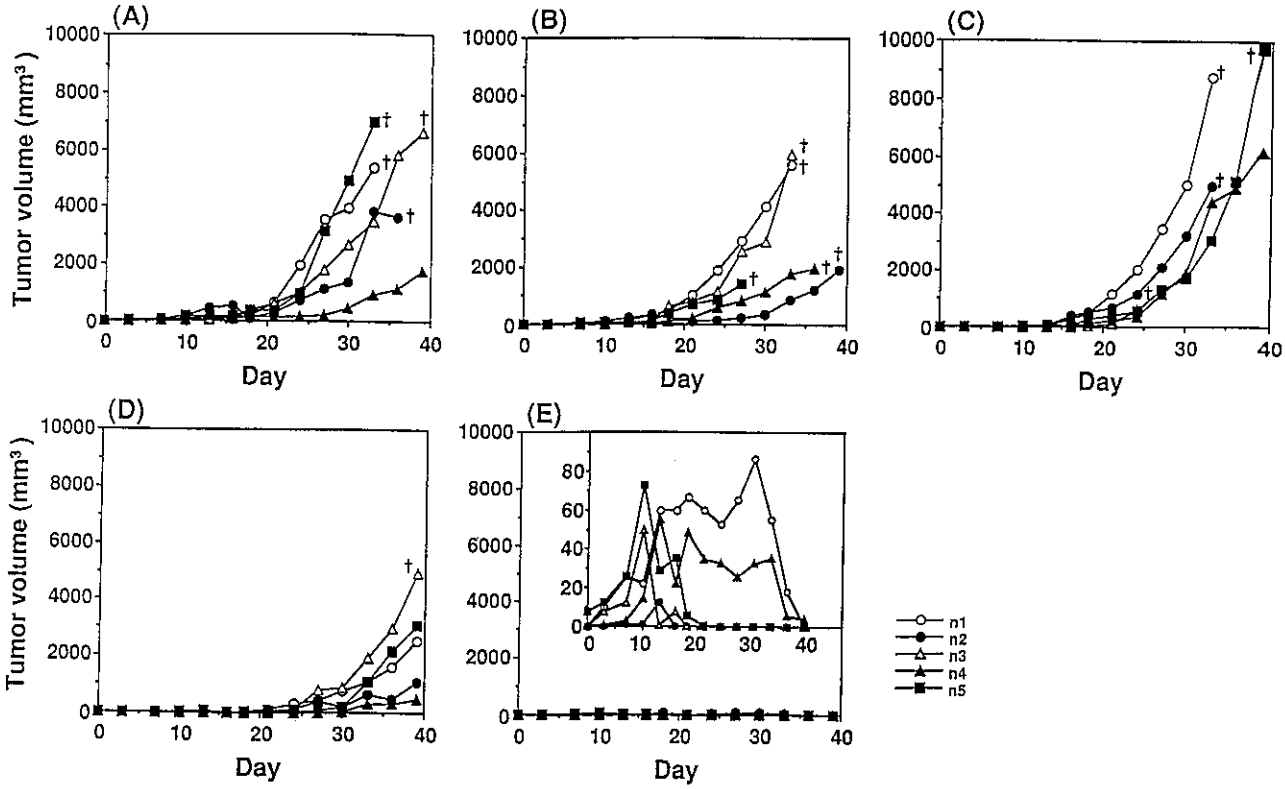

Fig. 1. Growth of unmodified and gene-modified tumor cells in mice. The tumor volume was measured after implantation of tumor cells (2×10^6) subcutaneously into the right flank of syngeneic BALB/c mice (n=5). Cells shown in panels are: (A); Meth-A (M0), (B); neo^R gene-transduced Meth-A (ML), (C); low TNF producer (E10), (D); moderate TNF producer (B1), and (E); high TNF producer (C5). Panel (E) shows the same results with an expanded scale. The notation † indicates death of the mouse.

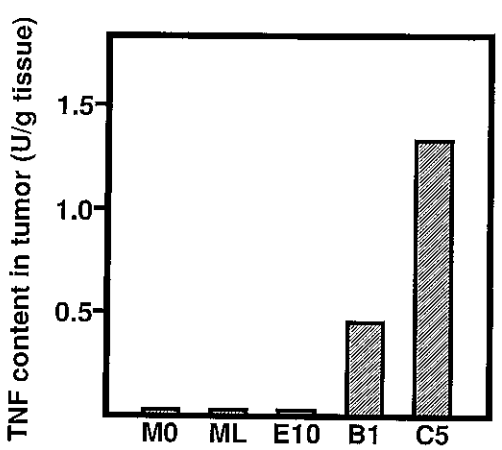

Fig. 2. TNF content in tumors developed from implanted MethA-TNF clones. Fourteen days after implantation, tumors developed from TNF gene-transduced Meth-A clones (E10, B1 and C5), neo^R gene-transduced Meth-A (ML), or parental Meth-A (M0) were removed surgically and each tumor tissue was minced in a homogenizer in PBS (pH 7.2). After centrifugation at 4000 rpm for 10 min, the supernatant was assayed for TNF concentration by the ELISA method.

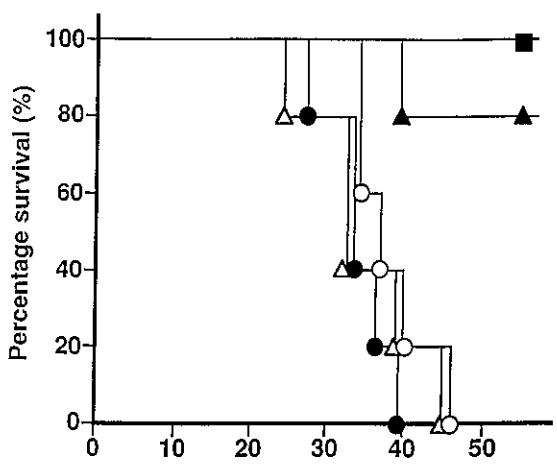

Fig. 3. Survival rate of mice implanted with unmodified and gene-modified tumor cells. Cells (2×10^6) were subcutaneously injected into the right flank of BALB/c mice (n=5). ○; unmodified Meth A (M0), ● neo^R gene-transduced Meth-A (ML), △; low TNF producer (E10), ▲; moderate TNF producer (B1), ■; high TNF producer (C5).

had undergone complete regression of previously inoculated C5 tumor (Fig. 4). These cells grew transiently, but then regressed, indicating that systemic immunity had been induced.

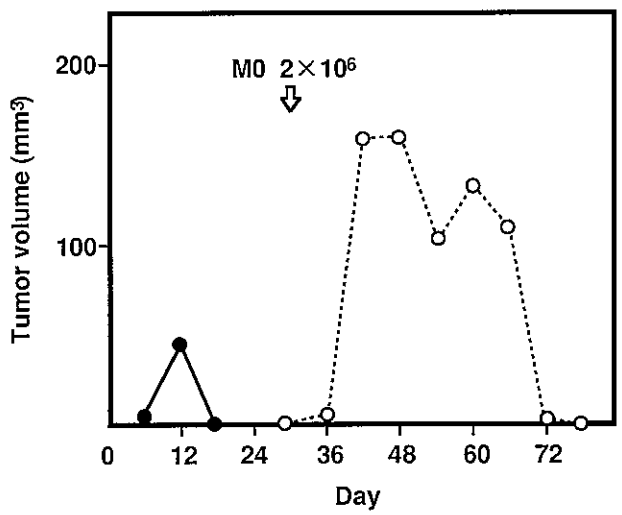

Fig. 4. Vaccinating effect of TNF-producing tumor. Cells of M0 (2×10^6) were inoculated into mice which had undergone complete regression of C5 tumor. ●; C5 tumor, ○; M0 tumor.

Augmented cytotoxic activity of CTLs and LAKs derived from splenocytes of mice implanted with TNF gene-transduced clone We then examined the cytotoxic activity of CTLs and LAKs from splenocytes of mice implanted with TNF gene-transduced clone (C5) and parental Meth-A (M0) cells, and that of normal mice, using a standard ^{51}Cr-release assay. As shown in Fig. 5, CTLs and LAKs derived from C5-bearing mice exhibited apparently higher cytotoxic activity against parental Meth-A than those from M0-bearing mice. Furthermore, C5 cells were much more sensitive to the same LAKs induced from tumor-bearing mice compared to M0 cells. LAKs from normal mice showed lower cytotoxic activities than those from mice implanted with M0 or C5. The CTL activities induced from normal mice against M0 were as low as those from M0-bearing mice, but were much lower than those from C5-bearing mice.

Population of freshly isolated splenocytes from mice bearing TNF gene-transduced Meth-A To clarify the reason for the augmentation of cytotoxic activity of CTL and LAK from splenocytes of mice implanted with TNF gene-transduced clone (C5), we examined the population of freshly isolated splenocytes by flow cytometry (Fig. 6).

In splenocytes from C5-bearing mice, T lymphocyte populations detected by anti-Thyl and Lyt1 Abs were significantly elevated over those of M0 (these Abs recognize pan-T antigens). In the T cell populations, Lyt2

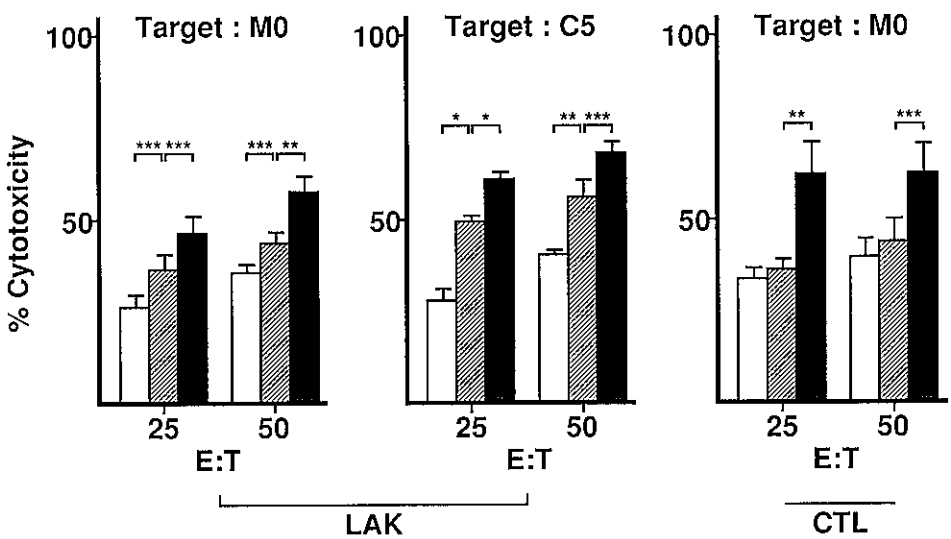

Fig. 5. Cytotoxic activities of LAK and CTL induced from splenocytes of mice implanted with unmodified Meth-A (M0) and high TNF producer (C5). Spleens from BALB/c mice implanted with M0 or C5 tumor cells (2×10^6), and those from normal mice were isolated, minced and cultured for induction of LAK and CTL, as described in "Materials and Methods." Cytotoxic activities of LAK and CTL against C5 and M0 were determined by ^{51}Cr-release assay. ■, ▨, □; LAK and CTL from spleen cells of C5-bearing, M0-bearing and normal mice, respectively. Each bar represents the mean±SD of three experiments. Data were analyzed statistically by the use of Student's t test. * $P<0.001$. ** $P<0.01$. *** $P<0.05$.

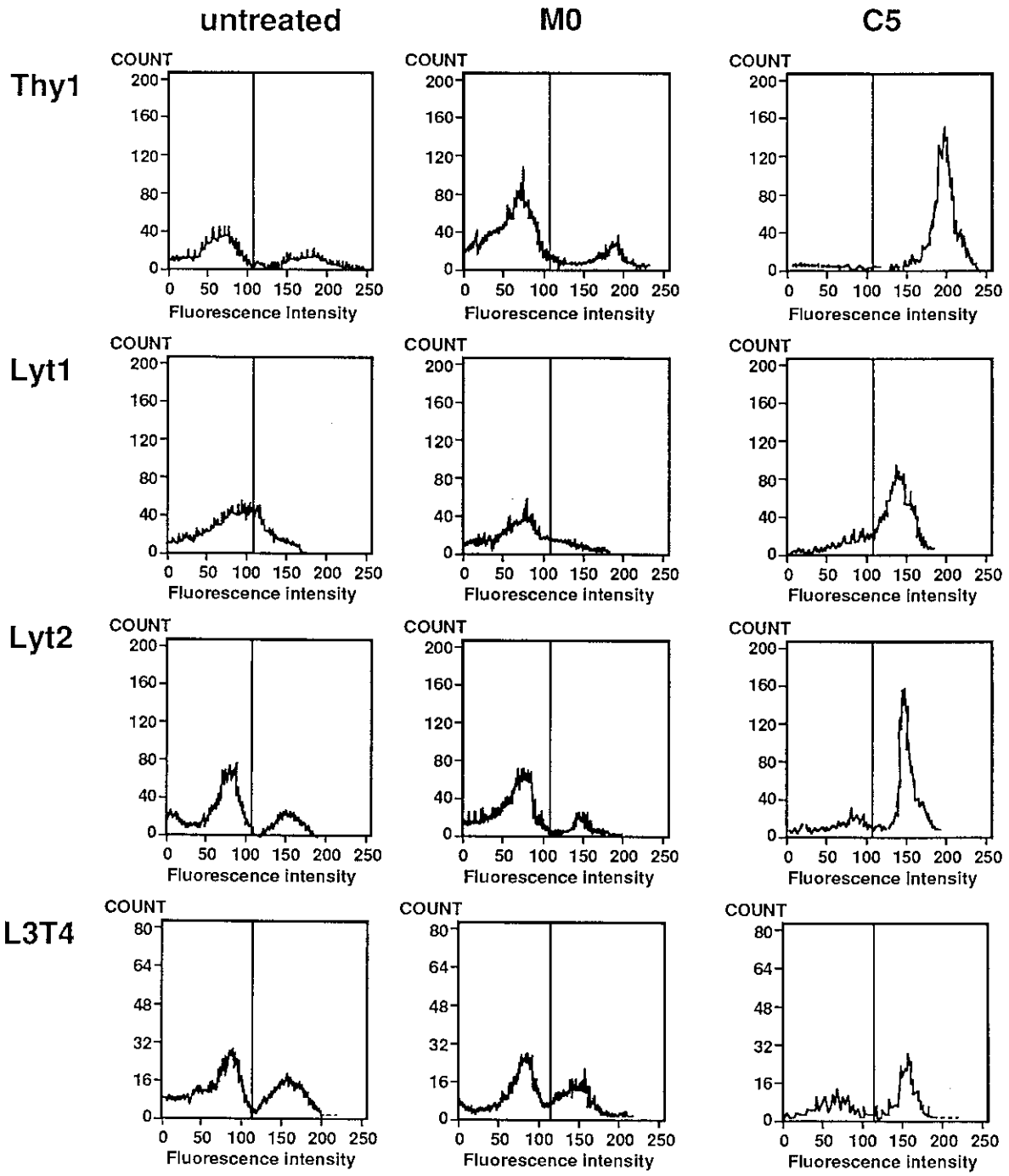

Fig. 6. Flow cytometric analysis of surface antigens of freshly isolated splenocytes from C5-bearing, M0-bearing and normal mice. Fluorescence intensity is indicated on a log scale. The count is the number of splenocytes. The vertical line in each figure is a marker of the cells stained positively.

(CD8)-positive T cells were especially increased as compared to L3T4 (CD4)-positive T cells: the percentages of Lyt2-positive T cells from M0- and C5-bearing mice were 6.0% and 73.9%, respectively, while the percentages of L3T4-positive T cells were 33.2% and 50.4%, respectively. As a control experiment, splenocytes from normal

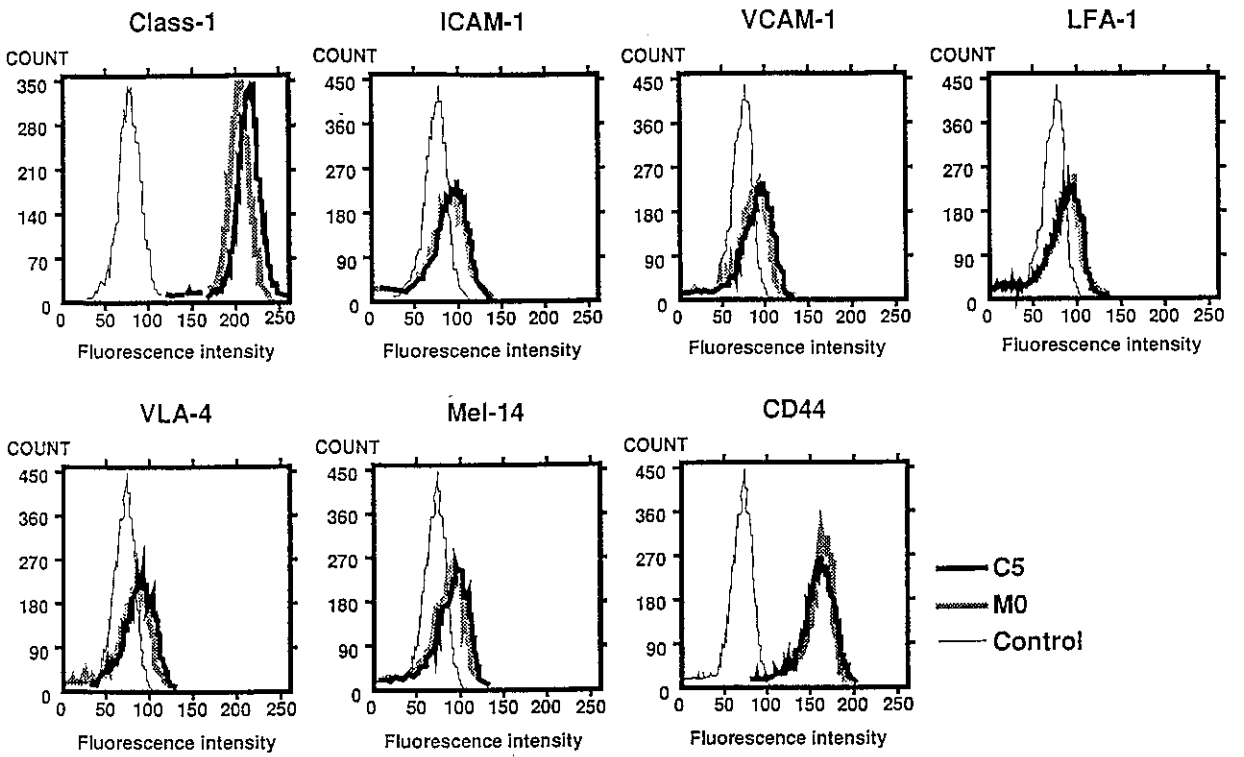

Fig. 7. Flow cytometric analysis of expression of MHC class-1 antigen and adhesion molecules on unmodified Meth-A (M0) and high TNF producer (C5) cells. Fluorescence intensity is indicated on a log scale. The count is the number of tumor cells.

mice were examined. The results were not significantly different from those of M0-bearing mice, with values of 7.0% and 32.0% for Lyt2 and L3T4, respectively.

Surface antigen expression of TNF gene-transduced Meth-A clone To elucidate whether the enhanced cytotoxicity of splenic LAK or CTL was due to increased immunogenicity of inoculated tumor cells, surface antigens on C5, including MHC class-1 antigen and adhesion molecules, were studied by flow cytometry and compared with those on M0 (Fig. 7). Although the fluorescence intensities of ICAM-1, VCAM-1, LFA-1, VLA-4, Mel-14 and CD 44 were not dissimilar between C5 and M0, a slight but significant increase of MHC class-1 antigen expression was observed in C5 as compared with M0.

DISCUSSION

In the present study, we firstly compared tumorigenicity of TNF gene-transduced tumor cells with that of non-gene modified tumor cells. We established three clones of TNF gene-transduced Meth-A fibrosarcoma cells which persistently produced different amounts of TNF (Table I). These established clones showed no differences in proliferation rate *in vitro* and did not exhibit any susceptibility at all to exogenous TNF treatment (unpublished observation). These results are compatible with our previous observations that the cytotoxicity was not brought about by insertion of TNF protein into cells[44] and that transduction of TNF gene into target cells resulted in the acquisition of refractory character to exogenous TNF.[32]

Although the *in vitro* proliferation rates of these clones were rather similar to each other, the growth rates of implanted tumors of these clones significantly varied; they were inversely correlated with the amount of TNF produced, and the tumor with the highest production of TNF (C5) underwent complete regression (Fig. 1). These results are consistent with previous reports.[30, 31, 45]

The decreased tumorigenicity of TNF-producing cells was actually reflected in the prolongation of survival rates of the mice (Fig. 3).

Furthermore, when parental tumor cells (M0) were inoculated to rechallenge mice that had undergone complete regression of previously inoculated C5 tumor, M0 was completely rejected after transient formation of a tiny tumor (Fig. 4), suggesting a vaccinating activity of TNF gene-modified cells. Such a prophylactic effect and the fact that TNF gene-transduction into Meth-A cells

per se did not alter the proliferation rate, as mentioned above, naturally led us to speculate that a host-mediated immune mechanism might be involved.

Asher *et al.*[30] claimed that T cell-mediated immunity was implicated in the reduced tumorigenicity of TNF gene-transduced tumors by demonstrating that depletion of $CD4^+$ or $CD8^+$ T cell subsets by administration of corresponding antisera resulted in recovery of tumorigenicity. On the other hand, Blankenstein *et al.*[31] suggested that regression of TNF gene-transduced tumor cells might be caused by activated inflammatory cells such as neutrophiles, macrophages or NK cells, since the administration of anti-CR3 mAb, which is known to block migration of these inflammatory cells, abrogated the regressive effect. Teng *et al.*[45] also concluded that T cell was not involved in this regression, on the basis of the observation that long-term inhibition of tumor growth was observed in the absence of T cell immunity (in nude mice).

Although in these reports the significance of immune responses in mice implanted with TNF gene-transduced tumor cells has been proved, no direct evidence of increased cytotoxic activity of immuno-responsive effector cells from the tumor-bearing host has been presented. Furthermore, it was not clear in those experiments whether the immune response was limited to tumor loci or was systemic. In order to generate a therapeutic effect on pre-existing tumors or a prophylactic effect, the "systemic" immunity, not the local immunity, must be enhanced.

In this context, we extended our study to examine "systemic" immune responses in C5-bearing mice that had undergone complete regression of the tumor, compared with those in M0-bearing mice; the results obtained clearly indicated the augmented killing activity of CTLs and LAKs derived from splenocytes of mice implanted with TNF-producing cells (C5) (Fig. 5). The activities of CTLs and LAKs from normal mice were as low as or less than those from M0-bearing mice, possibly because the splenocytes from normal mice were not stimulated by the tumor cells.

In addition, we examined populations of freshly isolated splenocytes by flow cytometry. In splenocytes from C5-bearing mice, T lymphocyte populations (Thy1-positive cells), particularly Lyt2 (CD8)-positive T cells, were significantly elevated as compared to those from M0-bearing mice (Fig. 6). This finding appears to suggest that $CD8^+$ cells play an important role in tumor regression in C5-bearing mice. The expressions of Thy1, Lyt1, Lyt2 and L3T4 on splenocytes of normal mice were as low as those of M0-bearing mice.

Augmentation of immunogenicity of TNF gene-transduced cells is plausible as one of the factors by which the host's "systemic" immunity was elevated. We therefore examined surface antigens of C5 by flow cytometry and found enhanced expression of MHC class-1 antigen on C5 compared with that on M0 (Fig. 7). Taking into account that tumor-associated antigen is presented by MHC class-1, it seems possible that increased expression of MHC class-1 antigen is responsible for elevation of the immunogenicity of TNF gene-transduced cells. Furthermore, the fact that the cytotoxicity of LAK against C5 was higher than that against M0 indirectly indicates enhanced immunogenicity of C5 cells.

With regard to the adhesion molecules, including ICAM-1, VCAM-1. LFA-1, VLA-4, Mel 14 and CD 44, we were not able to find any appreciable difference in expression between M0 and C5 (Fig. 7). However, this does not necessarily mean that these adhesion molecules do not play any role in the regression of implanted tumors, because secreted TNF may possibly induce them on tumor-surrounding tissues in a paracrine manner and thus attract the effector cells. In fact, in our preliminary study, we observed increased ICAM-1 and VCAM-1 expression on the extracellular matrix and endothelial cells in mice transplanted with C5 by immunohistochemical analysis (unpublished observation).

In summary, the present results suggest that induction of systemic immunity is one of the mechanisms of the reduced tumorigenicity of TNF gene-transduced tumor. Further, the increased systemic immunity can be ascribed to the increased immunogenicity of tumor cells. Thus, the approach of using TNF gene-modified tumor cells as vaccines appears to be promising for therapeutic and/or prophylactic application.

ACKNOWLEDGMENTS

We thank Mr. M. Yamaguchi (Kishimoto Biomedical Laboratory, Sapporo, Japan) for flow cytometry, Dr. Miyake (Department of Immunology, Saga Medical School, Japan) for making available anti-VCAM 1 mAb, and Mr. D. Day (English teacher at Sapporo Medical University) for linguistic advice.

(Received August 11, 1993/Accepted November 18, 1993)

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