Jpn. J. Cancer Res. 87, 757–764, July 1996

Transduction of the Macrophage Colony-stimulating Factor Gene into Human Multidrug Resistant Cancer Cells: Enhanced Therapeutic Efficacy of Monoclonal Anti-P-glycoprotein Antibody in Nude Mice

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To develop a therapeutic modality for overcoming multidrug-resistant (MDR) cancer with anti-MDR1 antibody, we examined the effect of macrophage colony-stimulating factor (M-CSF) gene transfection into MDR AD10 cells on therapy of MDR cancer with anti-MDR1 antibody (MRK17) in nude mice. MDR human ovarian cancer (AD10) cells were transduced with the human M-CSF gene inserted into an expression vector to establish gene-modified cells capable of producing low (ML-AD10), intermediate (MM-AD10) and high (MH-AD10) amounts of M-CSF. Systemic administration of MRK17 resulted in significant dose-dependent inhibition of subcutaneous growth of ML-AD10 tumors. In contrast, systemic administration of recombinant M-CSF in combination with MRK17 did not augment the therapeutic efficacy of MRK17 alone, but rather promoted the growth of the parent AD10 cells. To test the efficacy of *in vivo* M-CSF gene therapy combined with antibody, we mixed the parent AD10 cells with MH-AD10 cells producing a large amount of M-CSF, and inoculated the mixed cells subcutaneously. Treatment with MRK17 inhibited growth of the mixed cells more than that of the parent cells alone. Thus, combined therapy with anti-MDR1 mAb and M-CSF gene modification of MDR cancer cells may provide a new immunotherapeutic modality for overcoming MDR in humans.

Key words: Anti-P-glycoprotein antibody — Multidrug resistance — M-CSF — Gene therapy

Multidrug resistance (MDR) of tumors is a major obstacle to successful cancer chemotherapy. MDR1 (P-glycoprotein), one of the key molecules in MDR, has been shown to bind anti-cancer drugs,[1–3] and to function as an ATP-driven efflux pump for various cytotoxic drugs.[4,5] The expression of MDR1 was found to be elevated in intrinsically drug-resistant cancers as well as in some tumors that acquired drug resistance during chemotherapy.[6,7] Recently, even low levels of MDR1 expression were shown to serve as a marker of resistance to combination chemotherapy in human ovarian cancer and small cell lung cancer.[8] Thus, from an immunotherapeutic point of view, P-glycoprotein seems to be a good molecular target for the selective killing of tumor cells expressing it. There is encouraging evidence that murine monoclonal antibodies (mAbs) (MRK16, MRK17) raised specifically against human MDR1 induce lysis of MDR cancer cells *in vitro* by human effector cells,[9,10] and cause rapid regression of established s.c. MDR tumors, regression being complete in some animals.[11]

Macrophage colony-stimulating factor (M-CSF) is known to augment antibody-dependent monocyte-mediated cytotoxicity (ADCC) against cancer cells by increasing the number and affinity of Fc receptors expressed on the monocytes.[12–14] We recently reported that M-CSF-primed blood monocytes show enhanced ADCC in the presence of anti-MDR1 mAb against MDR human cancer cells expressing MDR1.[10] These findings indicated that one potentially important mechanism for the *in vivo* anti-tumor effect of M-CSF is its ability to augment monocyte-mediated anti-MDR1 mAb-dependent cytotoxicity against MDR cancer cells. To augment the *in situ* ADCC activity mediated by monocytes and macrophages, we inserted the M-CSF gene into human MDR AD10 cancer cells to establish M-CSF gene-modified cell lines with differing abilities to produce M-CSF, and found that tumor-derived M-CSF could augment anti-MDR1 mAb-dependent monocyte-mediated killing of MDR cancer cells.[15] Interestingly, we also found an inverse relationship between the ability of the cells to produce M-CSF and their tumorigenicity.[15] In this paper, we report the combined effects of M-CSF gene-transduction into MDR cancer cells and systemic administration of anti-MDR1 mAb in the therapy of human MDR cancer in nude mice.

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MATERIALS AND METHODS

Cell lines The human ovarian tumor A2780 and its adriamycin-resistant variant, 2780AD (AD10) cells, were kindly supplied by Drs. R. F. Ozols and T. C. Hamilton, National Cancer Institute. The characteristics of these cell lines have been reported.[16]

M-CSF gene-modified AD10 cells capable of producing various amounts of M-CSF were cloned as described previously.[15] Briefly, the pRc/CMV-MCSF plasmid was constructed by cloning a 1.8-kb *Eco*RI cDNA fragment containing the complete coding region of human M-CSF[17] into the *Hin*dIII cloning site of the pRc/CMV vector (Invitrogen, San Diego, CA). Transduction was performed by the calcium phosphate precipitation technique.[18] Selection of transfectants in RPMI 1640 with 10% FBS (fetal bovine serum) containing 500 μg/ml of G418 was started two days after transduction. G418-resistant colonies were pooled to yield a bulk transduced culture, which was then cloned by limiting dilution to yield several lines. All cell cultures were then tested for secretion of M-CSF. Tumor cell clones producing low (ML-AD10), intermediate (MM-AD10) and high (MH-AD10) levels of M-CSF and the parent AD10 cells were used in further experiments. For quantitative measurement of M-CSF production,[15] these cells (103 cells/ml) were incubated for 7 days and the supernatants were harvested. The amounts of M-CSF produced by the parent AD10, ML-AD10, MM-AD10 and MH-AD10 cells were <0.2, 1.6, 8.5 and 100 ng/ml, respectively.

Reagents FBS and G418 were purchased from Gibco (Grand Island, NY). A recombinant form of human M-CSF (specific activity, 0.8×10^6 U/mg protein) was obtained from Otsuka Pharmaceutical Co. (Tokushima). Anti-MDR1 mAb MRK17 (IgG1) was prepared as described previously.[3, 11] None of these materials contained endotoxins, as judged by amebocyte assay (Seikagaku Kogyo, Tokyo; minimal detection level 0.3ng/ml).

Isolation and culture of mouse peritoneal macrophages Peritoneal macrophages were harvested by lavage of the peritoneal cavity of nude mice with prewarmed PBS, and were washed twice with phosphate-buffered saline, and resuspended in CRPMI1640. The cells were then incubated in suspension in medium with or without human recombinant M-CSF for three days, resuspended in CRPMI1640 at various concentrations, and plated in 96-well Microtest III plates (Falcon, Oxford, CA). After one hour, nonadherent cells were removed by washing with the medium. At this point the purity of the macrophages was $>99\%$ as judged from their morphology and the result of nonspecific esterase staining. These macrophage-rich cultures were used as effector cells.

Antibody-dependent cell-mediated cytotoxicity (ADCC) The target cells were labeled with ^{51}Cr as described before.[9, 10] Various numbers of effector cells (macrophage monolayers) in 96-well Microtest III plates were mixed with suspensions (100 μl) of 1×10^4 ^{51}Cr-labeled target cells that had been incubated at 37°C for 30 min with various concentrations of anti-MDR1 antibody (MRK17). The plates were centrifuged for 3 min at 100 $\times g$, and then incubated for 4 h. The radioactivities of 100 μl samples of supernatant obtained by centrifugation were then counted in a gamma counter. Determinations were carried out in triplicate. The percentage of specific cytolysis was calculated from the release of ^{51}Cr from test samples and control samples as follows:

$$\%\ \text{Specific release} = (E - S) / (M - S) \times 100$$

where E is the release in the test sample (cpm in the supernatant from target cells incubated with effector cells and test antibody), S is the spontaneous release (cpm in the supernatant from target cells incubated with medium alone), and M is the maximum release (cpm released from target cells lysed with 1 *N* HCl). The spontaneous release observed with different target cells ranged from 5% to 17% (total lysis).

Determination of M-CSF M-CSF was measured by radioimmunoassay (sensitivity limit, 0.1 ng/ml) as described before.[19] Briefly, duplicate samples (100 μl) were mixed with ^{125}I-M-CSF (1000 cpm/100 μl) and then with a 2000-fold dilution of anti-M-CSF antiserum (200 μl). The anti-M-CSF antibody binding reaction attained equilibrium within 20 h at room temperature or within 48 h at 4°C. After incubation, bound antibody was separated from free ^{125}I-M-CSF by addition of 100 ml of normal rabbit serum diluted 400 times with PBS, 100 μl of anti-rabbit IgG serum diluted 40 times with PBS, and 1.0 ml of 6 % polyethylene glycol (MW 8000) in PBS. The tubes were shaken and centrifuged at 1000*g* for 15 min at 4°C. The supernatants were removed by aspiration, and the radioactivities of the precipitates were counted for 1 min in an automated gamma spectrometer.

Animal studies Female BALB/c-nu mice and AF-nu mice (3 and 8 weeks old) were purchased from Charles River Breeding Laboratories (Tokyo) and maintained at the Cancer Institute under standard conditions according to the Institutional Guidelines. The tumorigenic activities of control and M-CSF gene-transfected cells were assayed by injecting 0.1 ml of cell suspension s.c. into the right flank of 3-week-old mice (5–7 mice/group) via a 26-gauge needle on a 1-ml syringe. The parent AD10 cells were previously found to grow progressively in 69% of 3-week-old mice.[15] In preliminary experiments, we observed that when 2×10^7 parent AD10 cells were injected s.c. into 8-week-old nude mice, all the mice developed palpable tumors. In subsequent experiments, 8-week-old mice were used unless otherwise described. For therapy of s.c. tumors, 2×10^7 AD10 cells mixed with or without 2.4×10^5 or 7.2×10^5 MH-AD10 cells were inoculated into the right flank, and i.v. injections of MKR17 were

given on days 2 and 7 or days 9 and 14 after tumor inoculation. To test the effect of systemic administration of rM-CSF on the growth of AD10 cells, we injected 1 or 10 μg/ml of rM-CSF i.v. on days 8, 9, 10, 13, 14 and 15 with 10 μg/ml of MRK17 on days 9 and 14. Tumor growth was examined by palpation every day and the tumor volume was measured every 3–4 days for the indicated period. The experiment was carried out at least twice, producing similar results.

Statistical analysis The statistical significance of differences between groups was analyzed by the use of Student's *t* test (two-tailed).

RESULTS

Anti-MDR1 mAb-dependent killing of human MDR AD10 cells by murine peritoneal macrophages First, we examined whether M-CSF gene transduction of human MDR AD10 cells could affect their susceptibility to ADCC mediated by murine macrophages. The results are shown in Table I. Murine peritoneal macrophages were significantly cytotoxic to parent and M-CSF gene-modified AD10 cells at all E/T ratios tested in the presence of 0.1 μg/ml of MRK17 ($P<0.05$). There were no significant differences among the clones in their susceptibility to ADCC by murine macrophages.

Next, we tested whether human M-CSF could enhance anti-MDR1 mAb-dependent cytotoxicity mediated by murine macrophages. Peritoneal macrophages were incubated in the presence of 5000 U/ml of human M-CSF for 3 days and assayed for MRK17-dependent cellular cytotoxicity. Table II shows that treatment with human M-CSF of mouse peritoneal macrophages resulted in higher ADCC activity than that of medium-treated macrophages against both parent and M-CSF gene-transfected AD10 cells.

Effect of combined systemic injections of recombinant M-CSF and MRK17 on growth of AD10 cells First, we examined the effect of the time of treatment with MRK17 on palpable tumor formation by AD10 cells.

Table I. Anti-MDR1 mAb-dependent Killing of Human MDR AD10 Cells by Mouse Peritoneal Macrophages

E/T ratio	MRK17 (0.1 μg/ml)	% Specific cytotoxicity against[a]		
		Parent AD10	ML-AD10	MH-AD10
10	−	3.7±0.7[b]	2.8±0.9	3.7±1.8
	+	9.7±0.9[c]	7.3±2.3[c]	7.6±0.3[c]
20	−	9.2±0.4	8.7±1.1	5.8±1.0
	+	19.9±3.0[c]	19.9±2.3[c]	15.7±1.8[c]
40	−	16.6±2.6	18.5±1.5	11.3±1.6
	+	52.4±0.8[c]	47.5±3.8[c]	53.3±1.6[c]

a) Peritoneal macrophages of nude mice were assayed for anti-MDR1 mAb (MRK17)-dependent cytotoxicity against parent and M-CSF gene-modified MDR cancer cells at the indicated E/T ratios.
b) Mean±SD for triplicate cultures.
c) Significantly different from that for macrophages without MRK17 at the corresponding E/T ratio ($P<0.05$).

Table II. Anti-MDR1 mAb-dependent Killing of Human MDR AD10 Cells by Mouse Peritoneal Macrophages and Its Augmentation by M-CSF

MRK17 (0.1 μg/ml)	Treatment of macrophages[a]	% Specific cytotoxicity against		
		Parent AD10	ML-AD10	MH-AD10
−	Medium	2.6±3.0[b]	1.3±0.2	4.8±4.0
−	M-CSF	3.1±2.2	0.1±0.6	3.5±1.0
+	Medium	22.7±0.8	23.1±1.0	24.6±1.2
+	M-CSF	33.1±2.4[c]	31.3±1.6[c]	29.8±2.1[c]

a) Peritoneal macrophages of nude mice were incubated for 3 days in medium with or without the indicated concentrations of human M-CSF (5000 U/ml) before assay of anti-MDR1 mAb (MRK17)-dependent cytotoxicity against parent and M-CSF gene-modified MDR cancer cells at an E/T ratio of 20:1.
b) Mean±SD for triplicate cultures.
c) Significantly different from that for macrophages without M-CSF treatment in the presence of MRK17 ($P<0.05$).

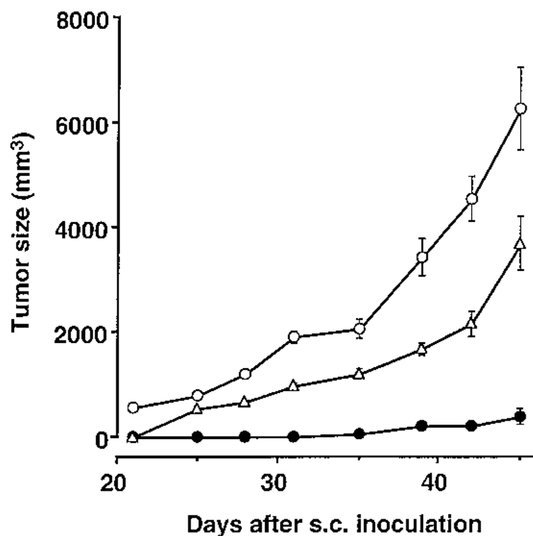

Fig. 1. Effect of time of treatment with MRK17 on s.c. growth of human MDR cancer (AD10) cells. All mice (6 or 7 mice/group) were inoculated s.c. with 2×10^7 AD10 cells on day 0 and then given i.v. injections of PBS (○) or 10 μg/ml of MRK17 (●) on days 2 and 7. One group of mice was also injected with 10 μg/ml of MRK17 on days 9 and 14 to test its growth inhibitory effect at a late phase (△). Bars represent mean±SE.

Table III. Effect of Combined Systemic Injections of M-CSF and Anti-MDR1 mAb (MRK17) on Growth of Human MDR AD10 Cell in Nude Mice

Treatment		Mice with tumor/total		Total (%)
		Exp. 1	Exp. 2	
PBS		6/6	6/6	12/12 (100)
M-CSF	1 μg	5/5	5/6	10/11 (91)
M-CSF	10 μg	6/6	6/6	12/12 (100)
MRK17	10 μg	5/6	4/6	9/12 (75)
M-CSF + MRK17 1 μg 10 μg		5/6	5/6	10/12 (83)
M-CSF + MRK17 10 μg 10 μg		6/6	5/6	11/12 (92)

All mice were injected s.c. in the right flank with 2×10^7 AD10 cells on day 0. On days 9 and 14 after tumor inoculation, groups of mice were treated i.v. with PBS or 10 μg of MRK17 and also treated with 1 or 10 μg of M-CSF on days 8, 9, 10, 13, 14 and 15. Tumor uptake was determined on day 56.

After s.c. inoculation of AD10 cells (2×10^7 cells), PBS with or without MRK17 (10 μg) was injected intravenously into 8-week-old mice on days 2 and 7, and also in some cases on days 9 and 14. The results are shown in Fig. 1. The parent AD10 cells formed palpable tumors in all mice and grew progressively. Under the same experimental conditions, treatment with MRK17 on days 2 and 7 resulted in almost complete inhibition of palpable tumor formation with 60% tumor uptake. When MRK17 was injected intravenously on days 9 and 14, moderate inhibition of tumor growth was seen in all the mice with palpable tumors.

Second, we examined whether combined treatment with MRK17 and rM-CSF instead of M-CSF gene transduction could be more effective therapeutically than either MRK17 or recombinant M-CSF alone for inhibition of tumor formation of AD10 cells inoculated s.c. into nude mice. For this, AD10 cells (2×10^7) were injected s.c. into nude mice, and then the mice were given i.v. injections of M-CSF on days 8, 9, 10, 13, 14 and 15 with or without MRK17 on days 9 and 14 after tumor inoculation. Tumor growth was examined by palpation for 40 days. Treatment with MRK17 alone resulted in 75% tumor formation by AD10 cells (Table III). Addition of recombinant M-CSF to MRK17 did not cause inhibition of tumor formation, but rather increased tumor uptake over that on treatment with MRK17 alone (Table III). When the sizes of growing tumors were also measured for 40 days, systemic treatment with MRK17 alone resulted in significant suppression of tumor formation by AD10 cells in nude mice, but that with recombinant M-CSF (10 μg) alone did not result in significant suppression ($P<0.05$). Moreover, recombinant M-CSF did not increase, but rather reduced the anti-tumor effect on growth of AD10 cells by MRK17 (Fig. 2).

Therapy of low-M-CSF-producing AD10 (ML-AD10) cells with anti-MDR1 mAb (MRK17) Our previous findings[15] showed that when human MDR cancer cells (2×10^7 cells/mouse) with or without M-CSF gene-modification were injected s.c. into 3-week-old nude mice, injection of parent AD10 cells or those transfected with a control gene resulted in palpable tumor outgrowth in 69% of the mice tested. ML-AD10 cells producing a small amount of M-CSF also formed palpable tumors in all recipient mice. MM-AD10 cells and MH-AD10 cells formed tumors in only 5 of 13 mice (38%), and 2 of 13 mice (15%), respectively. Under the same experimental conditions, we examined whether systemic administration of anti-MDR1 mAb (MRK17) could inhibit the s.c. growth of ML-AD10 cells producing a low amount of M-CSF in nude mice. For this, 2×10^7 cells were inoculated s.c. into mice, and on days 2 and 7, these mice received i.v. injections of MRK17 at concentrations of 3 to 100 μg/body. The tumor diameters were measured serially and the results are shown in Fig. 3. Intravenous administrations of MRK17 at doses of more than 3 μg resulted in significant inhibition of the growth of palpable tumors in a dose-dependent manner ($P<0.05$). Maximal inhibition of the subcutaneous growth of ML-AD10 cells

was seen in mice that received injections of 30 μg of MRK17 mAb.

Therapy by MRK17 of human MDR cancer consisting of a mixture of parent AD10 cells and MH-AD10 cells We examined whether gene-modification of a few cells within the growing MDR cancer was sufficient in treatment with MRK17 to inhibit growth of MDR cancer in nude mice. For this, we mixed the parent AD10 cells with MH-AD10 cells producing a large amount of M-CSF, inoculated the mixture s.c. into 8-week-old female nude

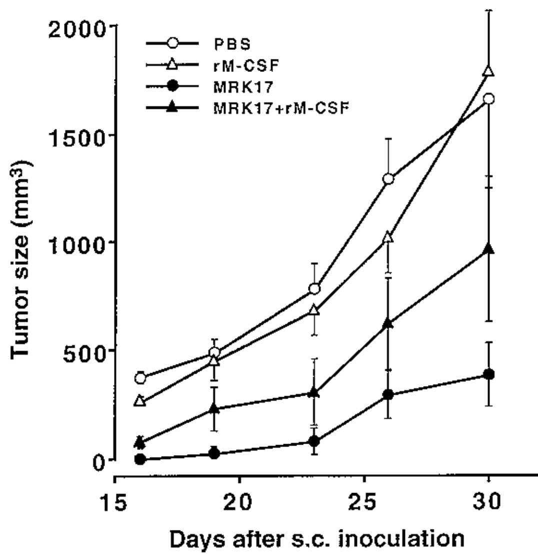

Fig. 2. Effect of combined systemic injections of recombinant human M-CSF and anti-MDR1 mAb (MRK17) on growth of human MDR AD10 cells in nude mice. All mice were injected s.c. in the right flank with 2×10^7 AD10 cells on day 0. On days 9 and 14 after tumor inoculation, groups of mice were treated i.v. with PBS or 10 μg of MRK17 and also treated with 10 μg of recombinant M-CSF (rM-CSF) on days 8, 9, 10, 13, 14 and 15. Bars represent mean±SE.

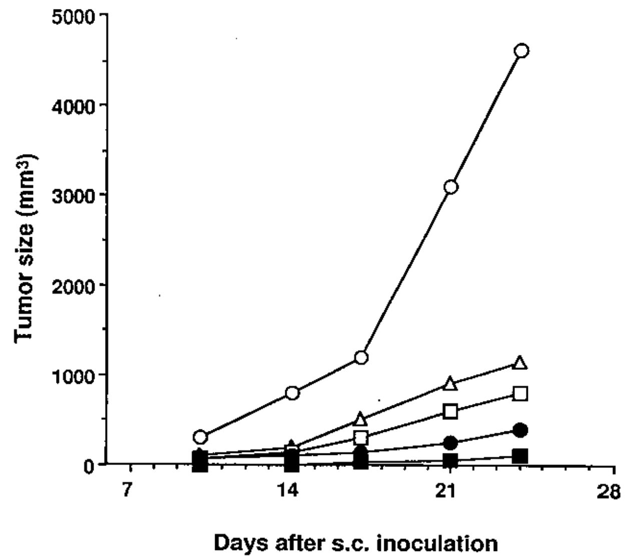

Fig. 3. Therapy of low-M-CSF-producing AD10 (ML-AD10) cells with anti-MDR1 mAb (MRK17). ML-AD10 cells (2×10^7 cells) were inoculated s.c. into groups of mice (6–7 mice/group), and on days 2 and 7, the mice were given i.v. injections of PBS (○) or MRK17 at a concentration of 3 (△), 10 (□), 30 (■) or 100 (●) μg/mouse. Tumor diameters were measured serially until day 24.

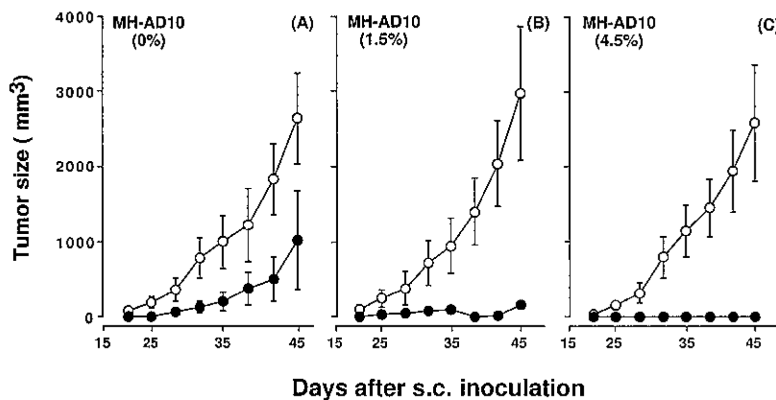

Fig. 4. Therapy with MRK17 of human MDR cancer consisting of a mixture of the parent AD10 cells and MH-AD10 cells. Mixtures of the parent AD10 cells (2×10^7) and the indicated percentages of AD10 cells producing a large amount of M-CSF (MH-AD10 cells) were inoculated s.c. into groups of mice (6 mice/group), and then PBS (○) or MRK17 (10 μg/body) (●) was injected on days 2 and 7. Tumor volumes were measured every 3–4 days until day 56. Bars represent mean±SE.

mice, and injected MRK17 (10 μg) on days 2 and 7. Results are shown in Fig. 4. When MRK17 was not administered, AD10 cells mixed with or without MH-AD10 cells grew progressively in all the mice. Systemic treatment with MRK17 (10 μg) significantly inhibited the growth of AD 10 cells alone after 28 days post tumor inoculation ($P<0.05$). Under the same experimental conditions, tumor formation of AD10 cells mixed with 1.5% or 4.5% of MH-AD10 cells was almost completely inhibited by MRK17 treatment as compared to that without mAb treatment.

DISCUSSION

In the present study, we demonstrated that M-CSF gene transduction into MDR AD10 cells, but not systemic injections of M-CSF, enhanced the therapeutic efficacy of anti-MDR1 mAb (MRK17) for human MDR cancer.

There is accumulating evidence that the use of antibodies may be effective in cancer treatment.[20] mAbs (MRK16 and MRK17) directed against P-gp expressed on MDR cancer cells are expected to be useful in the immunotherapy of human MDR malignant cells,[11] but possible side effects of anti-MDR1 antibodies administered to humans should be carefully studied because of MDR1 expression in normal tissues. We previously demonstrated that mAbs (MRK16 and MRK17) were effective in inducing human monocyte-mediated killing of MDR1-positive cancer cells and that M-CSF enhanced monocyte-mediated ADCC against human MDR cancer cells through enhancement of CD16, CD32 and CD64 expressions on monocytes,[10] suggesting an important anti-tumor role of M-CSF. The present study was designed to determine the therapeutic efficacy of M-CSF gene transduction into human MDR cancer cells in combination with anti-MDR1 mAb. In this study we found that human MDR ovarian cancer (AD10) cells growing subcutaneously in nude mice were susceptible to the systemic administration of anti-MDR1 mAb, and that MRK17 in combination with transduction of the M-CSF gene into MDR cancer cells was more therapeutically effective than either MRK17 or M-CSF gene transduction alone.

Macrophages are known to have dual roles in *in vivo* tumor growth and progression.[21–23] M-CSF was found to induce tumoricidal activity of monocytes.[13, 24] Moreover, administration of high doses of recombinant M-CSF was also shown to be effective in inducing tumor regression in experimental murine metastasis models.[25] We found that MH-AD10 cells capable of producing a large amount of M-CSF had greatly reduced tumorigenicity when the cells were inoculated s.c. in nude mice.[15] In contrast, gene-modified ML-AD10 cells capable of producing a small amount of M-CSF were found to form tumors in all mice examined, whereas the parent cells grew in only 69% of the recipients to yield palpable tumors.[15] The presence of a small amount of M-CSF at the tumor growth site might attract and influence the functions of tumor-associated macrophages which provide optimal micro-environment conditions for growth of AD10 tumors. Under these experimental conditions, treatment with MKR17 caused significant, dose-dependent eradication of ML-AD10 tumors (Fig. 3). This finding suggests that even if low levels of M-CSF are produced locally at the tumor site to promote tumor growth, combined use of anti-MDR1 antibody might be therapeutically useful for *in situ* destruction of MDR cancer cells.

We found that systemic administration of large amounts of recombinant human M-CSF was not effective in inhibiting the tumor formation of MDR ovarian cancer AD10 cells in nude mice, and that systemic administration of recombinant M-CSF in combination with anti-MDR1 mAb did not increase the anti-tumor effect on growth of AD10 cells inoculated s.c. in nude mice over that of anti-MDR1 mAb alone (Table III, Fig. 2). The reason for this failure is unknown at present. Nevertheless, there are several possible explanations. Firstly, systemic administration of exogeneous M-CSF might stimulate ovarian cancer growth *in vivo*, because a potential effect of exogeneous M-CSF on ovarian cancer cells has been reported in an *in vitro* model of the malignant phenotype of invasion.[25] Indeed, high levels of M-CSF in serum seem to imply a poor prognosis in patients with ovarian cancer.[26] Secondly, systemic administration of recombinant M-CSF might cause monocytosis in the circulation, but not allow monocytes to extravasate to the tumor site. Another possible explanation is that the lack of inhibitory effect of systemic M-CSF administration on the growth of s.c.- inoculated tumor cells might be due to insufficient M-CSF to stimulate macrophages at the local tumor site. This was not the case, however, in a metastatic model of melanoma because systemic administration of M-CSF reduced pulmonary or liver metastases.[27, 28] These findings suggest that systemic administration of M-CSF might reduce the metastatic spread of cancer cells because systemically activated monocyte-macrophages come in contact with cancer cells passing through the blood stream. MKR17 was therapeutically effective when given on days 2 and 7 after AD10 inoculation (Fig. 4), but it had little effect when given on days 9 and 14 (Fig. 2). Further study is required to find optimal conditions for combined treatment with recombinant M-CSF and anti-MDR1 antibody in terms of doses and timing of each agent.

Recently much attention has been paid to the development of methods for gene therapy of cancer.[29–32] In murine systems, there is encouraging evidence that vari-

ous methods of delivery of biologically active genes into animal cells are highly effective.[33–35] A successful gene delivery system may lead to gene therapeutic strategies for introduction of the M-CSF gene into MDR cancer cells which could be followed by treatment with anti-MDR1 mAb. With regard to gene targeting to cancer cells *in vivo*, even if an appropriate delivery system can be successfully designed, gene targeting does not seem to be 100% efficient in modifying the tumor cells. Presumably, some cancer cells can be gene-modified by a delivery system using physical or biological vectors. So, it is very important to know whether gene-modification of a few cells within a growing MDR cancer is sufficient for inhibition of growth of the MDR cancer in nude mice. To test this, we mixed the parent AD10 cells with MH-AD10 cells producing a large amount of M-CSF to provide the same level of M-CSF production as that by low-producing (ML-AD10) cells. For this, the mixed cells contained 1.5% and 4.5% MH-AD10 cells. All the recipient nude mice had palpable tumors even after s.c. inoculation of the parent AD10 cells with or without MH-AD10 cells. Treatment with MRK17 mAb was markedly more effective in inhibiting tumor formation of AD10 cells mixed even with 1.5% MH-AD10 cells than that of AD10 cells alone (Fig. 4). These findings strongly indicate that if a few cells within MDR tumors can be gene-targeted in a particular way to produce M-CSF, anti-MDR1 mAb (MRK17) should be therapeutically more useful for eradicating human MDR cancer.

In summary, local production of M-CSF at the site of tumor growth is a prerequisite for successful eradication of MDR ovarian cancer by anti-MDR1 mAb. The present findings suggest that combined treatment with M-CSF gene transduction and anti-MDR1 mAb should be effective against MDR cancer in humans.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan.

(Received January 22, 1996/Accepted April 19, 1996)

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