Jpn. J. Cancer Res. 87, 497–504, May 1996

Anti-ganglioside GM_2 Monoclonal Antibody-dependent Killing of Human Lung Cancer Cells by Lymphocytes and Monocytes

Masaki Hanibuchi, Seiji Yano, Yasuhiko Nishioka, Hiroaki Yanagawa and Saburo Sone[1]

Third Department of Internal Medicine, Tokushima University School of Medicine, 3 Kuramoto-cho, Tokushima 770

Ganglioside GM_2 (GM_2) frequently appears on the cell surface of human cancers of neuroendocrine origin. A mouse-human chimeric monoclonal antibody (mAb), KM966, against GM_2 was previously found to promote the lysis of various cancer cells by human blood mononuclear cells (MNC). In this study, we analyzed the effector cells responsible for the chimeric mAb-dependent cell-mediated cytotoxicity (ADCC) against small cell lung cancer (SCLC) cells and examined the enhancing effect of various cytokines on the ADCC activity. The ADCC activity was assessed by 4-h ^{51}Cr release assay. Highly purified lymphocytes (>99%) and monocytes (>90%) were separated by centrifugal elutriation from peripheral blood MNC of the same healthy donor. KM966 induced lysis of SCLC cells mediated by both lymphocytes and monocytes to similar extents, in a dose-dependent manner. Pretreatment of lymphocytes with various cytokines [interleukin (IL)-2, IL-12 and interferon-γ] and that of monocytes with macrophage-colony-stimulating factor significantly augmented the killer activity against SCLC cells in the presence of KM966 mAb. KM966 was also effective for the lysis of non-small cell lung cancer cells in direct proportion to the GM_2 expression levels. These findings suggest that combined treatment of KM966 mAb with cytokines may be therapeutically useful for *in vivo* killing of lung cancer cells expressing GM_2 through the ADCC reaction.

Key words: Ganglioside GM_2 — Chimeric antibody — KM966 — ADCC

Lung cancer is one of the major human cancers and has a poor prognosis,[1-4] since conventional therapies including chemotherapy, radiation therapy and surgical resection are not very effective against it. Therefore various new approaches to overcome lung cancer have been tried both *in vitro* and *in vivo*.[5-8] One of them is treatment with mAb[2] which recognizes an antigen specifically expressed on the surface of lung cancer cells.

GM_2 is widely expressed in human cancers of neuroendocrine or neuroectodermal origin, such as lung cancer,[9] melanoma,[10] glioblastoma and neuroblastoma,[11] but it is a minor ganglioside in normal tissues[10,11] and there are hints of a relationship to clinical features.[12-14] In some clinical trials, treatment with mAbs against various gangliosides induced regression of cutaneous melanoma and suppression of metastasis in neuroblastoma.[15-18] Human anti-GM_2 mAb (IgM type) has also been used in clinical studies, and some clinical response was reported.[16]

Recently the mouse-human chimeric anti-GM_2 mAb KM966 (IgG_1 type) was generated and found to react with and lyse various GM_2-positive human cancer cells, including lung cancer, through the ADCC reaction in the presence of human MNC.[9]

It is of interest to analyze the effector cell populations in the peripheral blood which are involved in chimeric mAb-mediated lysis of various cancer cells, because one potentially important mechanism for the *in vivo* anti-cancer effects of chimeric mAbs is their ability to mediate ADCC. It is likely that the type of effector cells which mediate chimeric mAb-dependent ADCC reaction varies depending on the nature of the antigens and mAbs.[19-21] Therefore, in this study, we performed detailed analysis of effector cells involved in KM966-dependent tumor cell lysis. Moreover, previous reports have demonstrated that the combined use of chimeric mAb and cytokines to augment the activity of the effector cells could induce higher cytolysis of cancer cells.[19,22] So, in this study, we also examined whether combined use of KM966 mAb and various cytokines is effective to augment KM966-dependent anti-cancer activities.

MATERIALS AND METHODS

Cell lines and cell culture The human small cell lung cancer SBC-3 cells were kindly provided by Dr. S. Hiraki (Okayama University, Okayama).[23] The human small

[1] To whom all correspondence should be addressed.

[2] Abbreviations: mAb, monoclonal antibody; GM_2, ganglioside GM_2; ADCC, antibody-dependent cell-mediated cytotoxicity; MNC, mononuclear cells; IL-2, interleukin-2; IL-12, interleukin-12; IFN-γ, interferon-γ; PBS, phosphate-buffered saline; M-CSF, macrophage-colony-stimulating factor; SCLC, small cell lung cancer; NK, natural killer; LAK, lymphokine-activated killer; MDR, multidrug-resistant; NSCLC, non-small cell lung cancer; HAMA, human anti-mouse antibody.

cell lung cancer H69 cells were obtained from the American Type Culture Collection (Rockville, MD).[24] The human lung adenocarcinoma RERF-LC-MS and the human lung squamous cell carcinoma RERF-LC-AI cells were kindly provided by Dr. M. Akiyama (Radiation Effects Research Foundation, Hiroshima).[25] The human lung adenocarcinoma PC-14 cells were kindly provided by Dr. N. Saijo (National Cancer Institute, Tokyo).[26] The human ovarian cancer A2780 cells were kindly supplied by Drs. R. F. Ozols and T. C. Hamilton (National Cancer Institute, Bethesda, MD).[27] Cell cultures were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum and gentamycin at 37°C in a humidified atmosphere of 5% CO_2 in air. Cytotoxicity assays were performed when the cultured target cells were in the exponential phase of growth.

Reagents Fetal bovine serum was purchased from M. A. Bioproducts (Walkerville, MD). The mouse-human IgG_1 chimeric anti-GM_2 mAb KM966[9] was purified as described previously. Recombinant human IL-2 (specific activity, 1.14×10^7 U/mg protein) was kindly provided by Takeda Pharmaceutical Co. (Osaka). Recombinant human IL-12 (specific activity, 5.26×10^6 U/mg protein) was supplied by the Genetics Institute (Cambridge, MA). M-CSF (specific activity, 1.0×10^7 U/mg protein) was supplied by Otsuka Pharmaceutical Co. (Tokushima). Recombinant human IFN-γ (specific activity, 5.36×10^6 U/mg) was a gift from Nippon Roche (Tokyo). None of these materials contained endotoxins, as judged by *Limulus* amebocyte assay (Seikagaku Kogyo Co., Tokyo: minimum detection level 0.1 ng/ml).

Analysis by flow microfluorometry Tumor cells were harvested and resuspended in PBS supplemented with 10% human pooled AB serum to prevent nonspecific antibody binding. After incubation for 30 min at 4°C, the cells were washed once and incubated for 30 min at 4°C in PBS containing KM966 (10 μg/ml) or mouse control serum (Tago, Inc., Burlingame, CA) (4 μg/ml). The cells were then washed with PBS, and fluorescein-conjugated goat anti-human IgG Fc (Organon Teknika Corp., West Chester, PA) was added as a second antibody. After incubation for 30 min at 4°C, they were washed again and the fluorescence intensity was measured with a FACScan (Becton Dickinson, Mountain View, CA).[28]

Isolation and culture of human lymphocytes and monocytes Leukocytes from peripheral blood (200 ml) of healthy donors were collected in an RS-6600 rotor of a Kubota KR-400 centrifuge, and MNC were separated from leukocytes in lymphocyte separation medium (Litton Bionetics, Kensington, MD). Lymphocytes and monocytes were separated from MNC by centrifugal elutriation in a Beckman JE-5.0 elutriation system.[29] Fractions enriched in lymphocytes (>99%) and in monocytes (>95%) were obtained at 3000 rpm and at flow rates of 26 ml/min and 30–36 ml/min, respectively. More than 97% of the cells were viable, as judged by the trypan blue dye exclusion test. The lymphocyte and monocyte fractions were each washed twice with PBS, and resuspended in the medium. The monocyte fraction was plated for 1 h in 96-well Microtest III plates (Falcon, Oxford, CA), and then non-adherent cells were removed by washing with medium. At this point the purity of the monocytes was >99% as judged from their morphology and nonspecific esterase staining.

Treatment of effector cells with various cytokines MNC of healthy donors were incubated for 4 days in medium with or without 1 U/ml of IL-2, 100 U/ml of IL-12 or 100 U/ml of IFN-γ. After 4 days, non-adherent cells were harvested as the lymphocyte fraction from MNC; the purity was >99% as judged from their morphology. Purified monocytes were incubated for 4 days in medium with or without 5000 U/ml of M-CSF. Then purified lymphocytes or monocytes (2.0×10^5/100 μl) were put into each well of 96-well Microtest III plates and ADCC assay was done as described below.

ADCC assay The target cells were labeled with ^{51}Cr as described before.[30] More than 97% of the cells were viable, based on the trypan blue dye exclusion test. Purified lymphocytes or monocytes (2.0×10^5/100 μl) in 96-well Microtest III plates were mixed with a suspension (100 μl) of 1.0×10^4 ^{51}Cr-labeled target cells with or without various concentrations of KM966. The plates were centrifuged for 3 min at 100*g*, and then incubated at 37°C for 4 h in a humidified 5% CO_2 atmosphere. After centrifugation, the radioactivity in 100 μl of supernatant was counted in a gamma counter. Determinations were carried out in triplicate. The percentage of specific cytolysis was calculated from the ^{51}Cr-releases from test and control samples, as follows:

$$\% \text{ specific lysis} = (E-S)/(M-S)\times100$$

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).

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

RESULTS

Characteristics of tumor cell lines used in this study Table I summarizes the reactivity of various human cancer cell lines to anti-GM_2 mAb KM966 analyzed by FACScan. Four of five lung cancer cell lines and the one

Table I. Characteristics of Cancer Cell Lines Used in This Study

Cell line	Origin	GM_2 expression (MFI[a])	
		Control[b]	KM966
SBC-3	Lung cancer (SCLC[c])	2.2	250.7
H69	Lung cancer (SCLC[c])	2.8	40.3
RERF-LC-MS	Lung cancer (Adeno.[d])	2.3	49.3
PC-14	Lung cancer (Adeno.[d])	2.0	45.6
RERF-LC-AI	Lung cancer (Sq.[e])	2.1	2.2
A2780	Ovarian cancer	2.9	137.1

a) Mean fluorescence intensity.
b) Control shows the MFI of cancer cells treated with second antibody alone.
c) Small cell lung cancer. *d*) Adenocarcinoma. *e*) Squamous cell carcinoma.

ovarian cancer cell line expressed GM_2 on their cell surfaces.

Effector cell analysis of ADCC induced by anti-GM_2 antibody KM966 against SCLC cells We analyzed effector cells of human peripheral blood responsible for the ADCC reaction. For this, lymphocytes and monocytes isolated from the same healthy donor were each incubated with ^{51}Cr-labeled SBC-3 cells in the absence or presence of various concentrations of KM966 for 4 h. In the absence of KM966, lymphocyte-mediated cytolytic activity (NK activity) and monocyte-mediated cytolytic activity were very low (5.5 ± 0.2 and 0.0 ± 0.7, respectively), but KM966 significantly induced the ADCC reaction mediated by both lymphocytes and monocytes against SBC-3 cells in a dose-dependent manner. The ADCC activities mediated by lymphocytes and monocytes induced by KM966 were similar, but at lower concentration of KM966 (0.01 μg/ml), the ADCC activity mediated by lymphocytes was significantly higher than that by monocytes (Fig. 1). From this dose titration curve, we determined that the optimal dose of KM966 was 1 μg/ml. Therefore, 1 μg/ml of KM966 was used in the subsequent experiments.

We also evaluated the effect of the E/T ratio on ADCC against SBC-3 cells. KM966 mAb (1 μg/ml) significantly induced the ADCC reaction mediated by lymphocytes and monocytes even at the E/T ratio of 5 (Fig. 2). The ADCC was significantly augmented in direct proportion to the E/T ratio.

Effects of pretreatment of human effector cells with various cytokines on KM966-mediated SCLC cell lysis Next, we examined the effect of various cytokines (IL-2, IL-12, IFN-γ and M-CSF) on lysis of SBC-3 cells by human effector cells with KM966. We have already demonstrated that the addition of monocytes to the lymphocyte cultures resulted in a significant increase in induction of lymphokine (IL-2, IL-12)-activated killer activity.[29,31] Therefore, in this experiment, we used MNC containing both lymphocytes and monocytes.

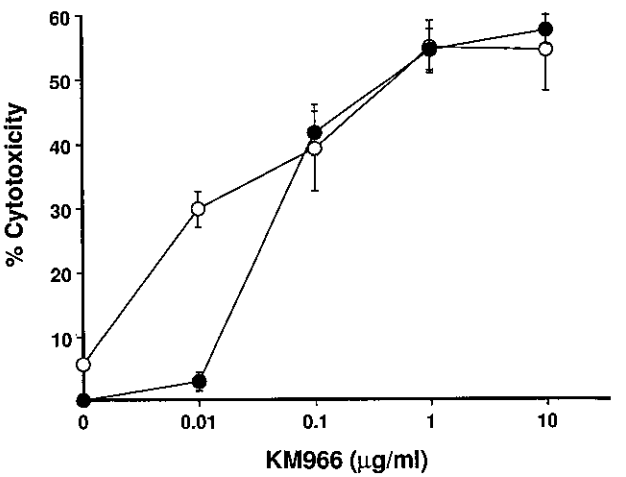

Fig. 1. Effect of KM966 on ADCC against SCLC cells. Lymphocytes (open circles) and monocytes (filled circles) were incubated for 4 h in medium with or without various concentrations of KM966 mAb with ^{51}Cr-labeled SBC-3 cells at an E/T ratio of 20, as described in "Materials and Methods." Bars show SDs of means for triplicate cultures. Data are representative of four separate experiments.

MNC were incubated for 4 days in medium with or without the optimal dose of IL-2 (1 U/ml),[29] IL-12 (100 U/ml)[31] or IFN-γ (100 U/ml).[32] As shown in Fig. 3, pretreatment of MNC with IL-2, IL-12 and IFN-γ significantly augmented their potential to lyse SBC-3 cells, and further enhancement of killer activity was observed on addition of KM966 at the effector phase. In a parallel experiment, monocytes were incubated for 4 days in medium with or without the optimal dose of M-CSF (5000 U/ml).[19] As shown in Fig. 4, the ADCC activity of monocytes cultured for 4 days in the absence of cytokines was significantly decreased, but M-CSF restored the ADCC activity of cultured monocytes to the level of that of freshly isolated monocytes (Table II).

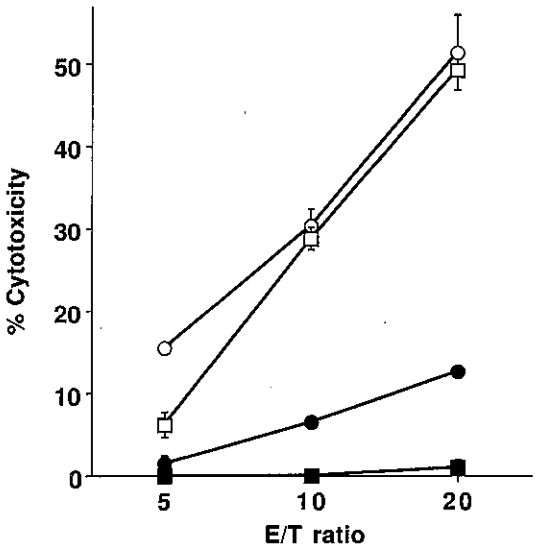

Fig. 2. Effect of E/T ratios on human lymphocyte- or monocyte-mediated cytolysis of SCLC cells. Lymphocytes (circles) or monocytes (squares) were incubated for 4 h in medium with (open symbols) or without (filled symbols) KM966 mAb (1 μg/ml) with ^{51}Cr-labeled SBC-3 cells at various E/T ratios, as described in "Materials and Methods." Bars show SDs of means for triplicate cultures. Data are representative of three separate experiments.

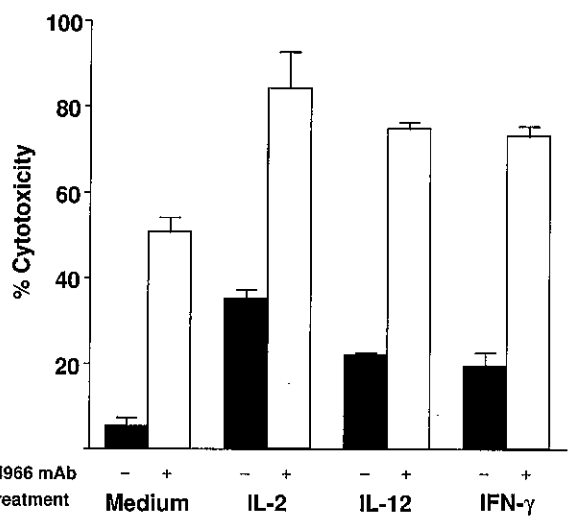

Fig. 3. Effect of pretreatment of human MNC with various cytokines on ADCC. Human MNC were incubated for 4 days in medium with or without IL-2 (1 U/ml), IL-12 (100 U/ml) or IFN-γ (100 U/ml), then non-adherent cells were harvested and washed. The resultant cells were incubated for 4 h in medium with (open columns) or without (filled columns) KM966 mAb (1 μg/ml) with ^{51}Cr-labeled SBC-3 cells at an E/T ratio of 20, as described in "Materials and Methods." Bars show SDs of means for triplicate cultures. Data are representative of three separate experiments.

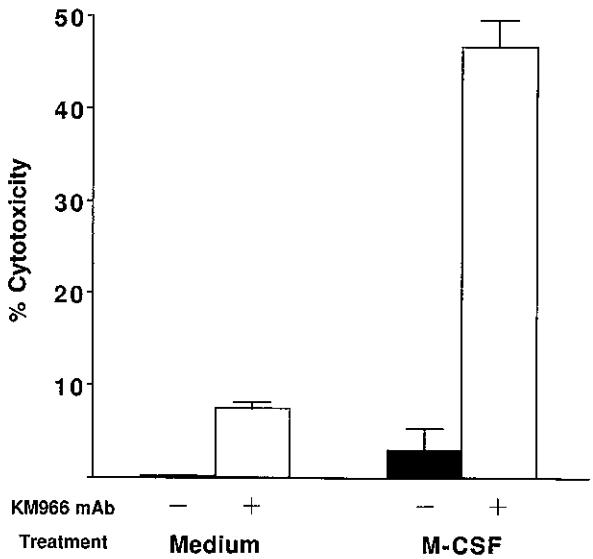

Fig. 4. Effect of pretreatment of human monocytes with M-CSF on ADCC. Human blood monocytes were incubated for 4 days in medium with or without M-CSF (5000 U/ml) and then harvested. The resultant cells were incubated for 4 h in medium with (open columns) or without (filled columns) KM966 mAb (1 μg/ml) with ^{51}Cr-labeled SBC-3 cells at an E/T ratio of 20, as described in "Materials and Methods." Bars show SDs of means for triplicate cultures. Data are representative of three separate experiments.

Effect of KM966 on human effector cell-mediated cytolysis against various human cancer cell lines We investigated the effect of KM966 on ADCC against various human cancer cell lines. KM966 induced both lymphocyte- and monocyte-mediated cytolysis of H69 (SCLC), RERF-LC-MS (lung adenocarcinoma), PC-14 (lung adenocarcinoma) and A2780 (ovarian cancer) cells, but not RERF-LC-AI (lung squamous cell carcinoma) cells (Table II). The GM_2 expression levels of the cancer cells used in this study were significantly correlated with the ADCC activity mediated by both lymphocytes and monocytes against these cancer cells ($r=0.82$, $P<0.05$ and $r=0.97$, $P<0.01$, respectively, data not shown).

Evaluation of specificity of KM966 mAb for cancer cell killing Finally, we evaluated whether KM966-dependent cytolysis is specific to GM_2-positive cancer cells. A suspension (50 μl) of 1.0×10^4 ^{51}Cr-labeled SBC-3 (GM_2-positive) cells or RERF-LC-AI (GM_2-negative) cells was mixed with a suspension (50 μl) of unlabeled RERF-LC-AI cells or SBC-3 cells, respectively; the labeled/unlabeled cell ratios were 0.5, 1 and 2 in each case. Human effector cell-mediated cytotoxicity was measured with or without KM966 mAb as described above. As shown in Fig. 5A, the lymphocyte-mediated killing of ^{51}Cr-labeled RERF-LC-AI cells (GM_2-negative) was not significantly

augmented by the addition of unlabeled SBC-3 cells (GM_2-positive) irrespective of the presence of KM966 mAb. Similarly, ADCC of ^{51}Cr-labeled SBC-3 cells was not significantly inhibited by the addition of unlabeled RERF-LC-AI cells (Fig. 5B). The results of monocyte-mediated killing were similar (Fig. 5, A and B).

Table II. Effect of KM966 on Human Lymphocyte- or Monocyte-mediated Cytotoxicity against Various Human Cancer Cell Lines

Target cells	KM966 (1 μg/ml)	% Cytotoxicity[a]	
		Lymphocyte	Monocyte
SBC-3	−	5.5±0.2[b]	2.9±0.3
	+	54.9±4.1[c]	49.1±1.0[c]
H69	−	14.5±6.3	4.9±2.1
	+	31.7±3.4[c]	26.0±2.9[c]
RERF-LC-MS	−	0.0±1.0	0.4±1.6
	+	25.5±4.0[c]	27.4±1.1[c]
PC-14	−	15.7±0.4	1.8±1.1
	+	46.8±0.3[c]	29.9±1.0[c]
RERF-LC-AI	−	1.7±1.2	0.0±0.7
	+	3.1±0.5	0.6±1.0
A2780	−	9.7±1.0	0.8±0.6
	+	59.2±0.0[c]	54.4±0.8[c]

a) Lymphocytes or monocytes were incubated for 4 h in medium with or without KM966 mAb (1 μg/ml) with ^{51}Cr-labeled cells at an E/T ratio of 20, as described in "Materials and Methods."
b) Mean±SD for triplicate cultures. Data are representative of four separate experiments.
c) Significantly different from the value in the absence of KM966 mAb ($P<0.01$).

DISCUSSION

Our study shows that chimeric KM966 mAb can induce the ADCC reaction mediated by both lymphocytes and monocytes against lung cancer cells in direct proportion to the GM_2 expression levels and that some cytokines can augment the cytotoxic activity mediated by both lymphocytes and monocytes against SCLC cells.

Previous studies have suggested that the types of effector cells which mediate chimeric mAb-dependent ADCC reaction vary depending on the nature of the recognized antigen and mAbs.[19–21] For example, a chimeric mAb ch14.18 with specificity for tumor-associated antigen was found to induce ADCC by granulocytes more efficiently than that by NK cells, and to have no effect on monocytes.[21] Masucci *et al.* reported that monocytes were major effectors in chimeric 17-1A mAb-mediated lysis of colorectal carcinoma cells.[20] We have also demonstrated that a chimeric mAb MH162 directed to P-glycoprotein had higher potential than its all-mouse counterpart MRK16 to induce lymphocyte-mediated lysis of MDR ovarian cancer (AD10) cells.[19] In this study, KM966 directed to GM_2 was found to induce ADCC mediated by both lymphocytes and monocytes against lung cancer cells. Although both lymphocytes and monocytes can be effector cells for KM966-dependent cancer cell lysis, we found that the ADCC activity with lymphocytes is significantly higher than that with monocytes at a suboptimal concentration of KM966 (0.01 μg/ml), as shown in Fig. 1. This is an important point, because it is difficult to maintain the optimum concentration of mAb *in vivo*. Therefore, lymphocytes may be the major effector cells which induce KM966-

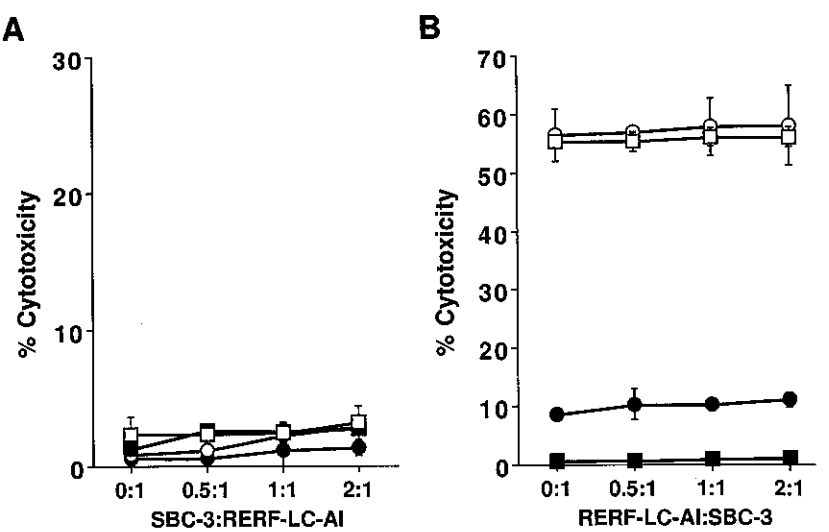

Fig. 5. Evaluation of specificity of KM966-dependent human lymphocyte- or monocyte-mediated GM_2-positive cancer cell killing. Lymphocytes (circles) or monocytes (squares) were incubated for 4 h in medium with ^{51}Cr-labeled RERF-LC-AI cells and unlabeled SBC-3 cells (A) or ^{51}Cr-labeled SBC-3 cells and unlabeled RERF-LC-AI cells (B) at labeled/unlabeled cell ratios of 0.5, 1 and 2 in the presence (open symbols) or absence (filled symbols) of KM966 mAb (1 μg/ml). Bars show SDs of means for triplicate cultures. Data are representative of three separate experiments.

dependent cytolysis *in vivo*. In addition, KM966 seems to be effective under physiological conditions, because the present study has also shown that KM966 can induce ADCC by both lymphocytes and monocytes even at the E/T ratio of 5 (Fig. 2).

Previous reports have demonstrated that GM_2 is widely and strongly expressed in human cancer cells including not only SCLC,[9] but also NSCLC.[33] Miyake *et al.* have reported that among the glycolipid preparations extracted from the lung cancer tissues, a significant amount of GM_2 was detected in 70% of the squamous cell carcinoma cases, 50% of the adenocarcinoma cases, 33% of the large cell carcinoma cases and 100% of the SCLC cases.[33] The present results show that GM_2 is expressed on various cancer cell lines (Table I) and that KM966 can induce ADCC against GM_2-positive cancer cells (Table II). Moreover, the human lymphocyte- and monocyte-mediated killing of GM_2-negative RERF-LC-AI cells is not significantly augmented by the addition of GM_2-positive SBC-3 cells in the presence of KM966 mAb (Fig. 5A). These results suggest that KM966-dependent cancer cell lysis is highly specific for GM_2-positive cancer cells, irrespective of the cell type.

The combined use of chimeric mAb and cytokines to augment the activity of the effector cells could induce higher cytolysis of cancer cells.[19, 22] In this study, the ADCC activity of monocytes cultured for 4 days in the absence of cytokines was significantly decreased (Fig. 4) compared with that of freshly isolated monocytes (Table II) due to the down-regulation of Fc receptor expression of monocytes during *in vitro* incubation.[19, 34] But, upon addition of M-CSF to the culture medium, the ADCC activity of cultured monocytes recovered to the level of that of freshly isolated monocytes (Fig 4. and Table II). The mechanism of the augmentation may be the increase of Fc receptor expression by M-CSF.[35] Our results indicated that M-CSF restored the ADCC activity of cultured monocytes to the level of that of freshly isolated monocytes. Moreover, in this study, the killer activity of lymphocytes was also augmented by the addition of cytokines, such as IL-2, IL-12 and IFN-γ, irrespective of the addition of KM966 (Fig. 3). Because IL-2, IL-12 and IFN-γ are well known to induce LAK activity against cancer cells[29, 31, 36, 37] through the induction of killing molecules such as perforin, the augmentation of the killer activity by these cytokines may arise mainly via the induction of killing molecules. These findings suggest that the combined use of KM966 mAb and cytokines may be successful for eradication of GM_2-positive cancer cells.

Besides the induction of the ADCC activity as mentioned above, KM966 mAb has other advantages. GM_2 is a minor ganglioside in normal tissues.[10, 11] Therefore KM966 mAb can hardly lyse normal cells, but nevertheless can induce specific lysis of GM_2-positive cancer cells through the ADCC reaction. Moreover, because KM966 is a chimeric mAb, the therapeutic use of this chimeric mAb should be less likely to cause HAMA responses than the use of its all-murine counterpart. Recent clinical reports on phase I studies using chimeric mAbs have demonstrated marked reduction of the HAMA responses to the extent that repeated injections are feasible,[38, 39] with sufficient prolongation of the serum half-life.

For effective immunotherapy of cancer, the biodistribution and specificity of mAb to cancer tissues are very important. Hanai *et al.* have demonstrated an *in vivo* therapeutic effect of KM966 mAb against GM_2-positive human lung cancer cells.[9] Intravenously administered KM966 mAb completely inhibited the growth of s.c. inoculated SBC-3 cells in nude mice, indicating that KM966 mAb may be distributed in sites of tumor growth, and may be suitable for clinical application. We have started to study the *in vivo* therapeutic effect of KM966 mAb at the organ level using a metastasis model of SBC-3 cells in SCID mice.

Further studies are needed before clinical application of KM966 mAb. In particular, we need to know 1) whether effector cells of cancer patients can mediate KM966-dependent cytolysis of autologous cancer cells, 2) whether combined use of KM966 with cytokines can facilitate the ADCC reaction mediated by effector cells of cancer patients and 3) how we can evaluate the expression of GM_2 in cancer patients.

ACKNOWLEDGMENTS

This work was supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan. We thank F. Kaneko for assistance in the preparation of lymphocytes and monocytes.

(Received November 6, 1995/Accepted January 29, 1996)

REFERENCES

1) Ries, L. G., Pollack, E. S. and Young, J. L. Cancer patient survival: surveillance, epidemiology and end results program, 1973–79. *J. Natl. Cancer Inst.*, **70**, 693–707 (1983).
2) Paesmans, M., Sculier, J. P., Libert, P., Bureau, G., Dabouis, G., Thiriaux, J., Michel, J., Van Cutsem, O., Sergysels, R., Mommen, P. and Klastersky, J. Prognostic factors for survival in advanced non-small-cell lung cancer: univariate and multivariate analyses including re-

cursive partitioning and amalgamation algorithms in 1,052 patients. *J. Clin. Oncol.*, **13**, 1221–1230 (1995).
3) Sufarlan, A. W. and Zainudin, B. M. Combination chemotherapy for small cell lung cancer. *Med. J. Malaysia*, **48**, 166–170 (1993).
4) Blackstein, M. E. Advances in chemotherapy for small cell lung cancer. *Semin. Oncol.*, **21** (Suppl. 1), 38–42 (1994).
5) Yang, S. C., Owen-Schaub, L., Grimm, E. A. and Roth, J. A. Induction of lymphokine-activated killer cytotoxicity with interleukin-2 and tumor necrosis factor-alpha against primary lung cancer targets. *Cancer Immunol. Immunother.*, **29**, 193–198 (1989).
6) Vangsted, A. J. and Zeuthen, J. Monoclonal antibodies for diagnosis and potential therapy of small cell lung cancer — the ganglioside antigen fucosyl-GM1. *Acta Oncol.*, **32**, 845–851 (1993).
7) Lynch, T. J. Immunotoxin therapy of small-cell lung cancer. N901-blocked ricin for relapsed small-cell lung cancer. *Chest*, **103** (Suppl. 4), 436–439 (1993).
8) Lissoni, P., Meregalli, S., Fossati, V., Paolorossi, F., Barni, S., Tancini, G. and Frigerio, F. A randomized study of immunotherapy with low-dose subcutaneous interleukin-2 plus melatonin vs chemotherapy with cisplatin and etoposide as first-line therapy for advanced non-small cell lung cancer. *Tumori*, **80**, 464–467 (1994).
9) Nakamura, K., Koike, M., Shitara, K., Kuwana, Y., Kiuragi, K., Igarashi, S., Hasegawa, M. and Hanai, N. Chimeric anti-ganglioside GM_2 antibody with antitumor activity. *Cancer Res.*, **54**, 1511–1516 (1994).
10) Portoukalian, J., Zwingelstein, G. and Dore, J. Lipid composition of human malignant melanoma tumors at various levels of malignant growth. *Eur. J. Biochem.*, **94**, 19–23 (1979).
11) Yates, A. J., Thompson, P. K., Boesel, C. P., Albrightson, C. and Hart, R. W. Lipid composition of human neural tumors. *J. Lipid Res.*, **20**, 428–436 (1979).
12) Pukel, C. S., Lloyd, K. O., Travassor, R., Dippold, W. G., Oettgen, H. F. and Old, L. J. GD_3, a prominent ganglioside of human melanoma. *J. Exp. Med.*, **155**, 1133–1147 (1982).
13) Ravindranath, M. H., Tsuchida, T., Morton, D. L. and Irie, R. F. Ganglioside GM_3:GD_3 ratio as an index for the management of melanoma. *Cancer*, **67**, 3029–3035 (1991).
14) Jennemann, R., Rodden, A., Bauer, B. L., Mennel, H. D. and Wiegandt, H. Glycosphingolipids of human gliomas. *Cancer Res.*, **50**, 7444–7449 (1990).
15) Cheung, N. V., Lazarus, H. and Miraldi, F. D. Ganglioside GD_2 specific monoclonal antibody 3F8: a phase-I study in patients with neuroblastoma and malignant melanoma. *J. Clin. Oncol.*, **5**, 1430–1440 (1987).
16) Irie, R. F., Matsuki, T. and Morton, D. L. Human monoclonal antibody to ganglioside GM_2 for melanoma treatment. *Lancet*, **i**, 786–787 (1989).
17) Vadhan-Raj, S., Cordon-Cardo, C., Carswell, E., Mintzer, D., Dantis, L., Duteau, C., Tempelton, M. A., Oettgen, H. F., Old, L. J. and Houghton, A. N. Phase-I trial of a mouse monoclonal antibody against GD_3 ganglioside in patients with melanoma: induction of inflammatory responses at tumor sites. *J. Clin. Oncol.*, **6**, 1636–1648 (1988).
18) Saleh, M. N., Khazaeli, M. B., Wheeler, R. H., Dropcho, E., Lui, T., Urist, M., Miller, D. M., Lawson, S., Dixton, P., Russel, C. H. and LoBuglio, A. F. Phase I trial of the murine monoclonal anti-GD_2 antibody $14G2_a$ in metastatic melanoma. *Cancer Res.*, **52**, 4342–4347 (1992).
19) Nishioka, Y., Sone, S., Heike, Y., Hamada, H., Ariyoshi, K., Tsuruo, T. and Ogura, T. Effector cell analysis of human multidrug-resistant cell killing by mouse-human chimeric antibody against P-glycoprotein. *Jpn. J. Cancer Res.*, **83**, 644–649 (1992).
20) Masucci, G., Wersall, P., Ragnhammer, P. and Mellstedt, H. Granulocyte-monocyte-colony-stimulating factor augments the cytotoxic capacity of lymphocytes and monocytes in antibody-dependent cellular cytotoxicity. *Cancer Immunol. Immunother.*, **29**, 288–292 (1989).
21) Barker, E., Mueller, B. M., Handgretinger, R., Herter, M., Yu, A. L. and Reisfeld, R. A. Effect of a chimeric anti-ganglioside GD_2 antibody on cell-mediated lysis of human neuroblastoma cells. *Cancer Res.*, **41**, 144–149 (1991).
22) Orlandi, R., Figini, M., Tomasetti, A., Canevari, S. and Colnaghi, M. I. Characterization of a mouse-human chimeric antibody to a cancer-associated antigen. *Int. J. Cancer*, **52**, 588–593 (1992).
23) Yonei, T., Ohnoshi, T., Hiraki, S., Ueoka, H., Kiura, K., Moritaka, T., Shibayama, T., Tabata, M., Segawa, Y. and Takigawa, N. Antitumor activity of platinum analogs against human lung cancer cell lines and tumor specimens. *Acta Med. Okayama*, **47**, 233–241 (1993).
24) Mirski, S. E., Gerlach, J. H. and Cole, S. P. Multidrug resistance in a human small cell lung cancer cell line selected in adriamycin. *Cancer Res.*, **47**, 2594–2598 (1987).
25) Teraoka, S., Kyoizumi, S., Seyama, T., Yamakido, M. and Akiyama, M. SCID mice model for the *in vivo* study of human oncotherapy: studies on the growth and metastasis of human lung cancer. *Int. J. Oncol.*, **5**, 501–508 (1994).
26) Ohe, Y., Nakagawa, K., Fujiwara, Y., Sasaki, Y., Minato, K., Bungo, M., Niimi, S., Horichi, N., Fukuda, M. and Saijo, N. *In vitro* evaluation of the new anticancer agents KT6149, MX-2, SM5887, menogaril, and liblomycin using cisplatin- or adriamycin-resistant human cancer cell lines. *Cancer Res.*, **49**, 4098–4102 (1989).
27) Hamada, H., Okochi, E., Oh-hara, T. and Tsuruo, T. Purification of the Mr 22,000 calcium-binding protein (sorcin) associated with multidrug resistance and its detection with monoclonal antibodies. *Cancer Res.*, **48**, 3173–3178 (1988).
28) Maeda, K., Sone, S., Ohmoto, Y. and Ogura, T. A novel differentiation antigen on human monocytes that is specifically induced by granulocyte-macrophage colony-stimulating factor or IL-3. *J. Immunol.*, **146**, 3779–3784 (1991).

29) Sone, S., Inamura, N., Nii, A. and Ogura, T. Heterogeneity of human lymphokine (IL-2)-activated killer (LAK) precursors and regulation of their LAK induction by blood monocytes. *Int. J. Cancer,* **42,** 428–434 (1988).
30) Yano, S., Sone, S., Nishioka, Y., Naito, M., Tsuruo, T. and Ogura, T. Cyclosporin A enhances susceptibility of multidrug resistant human cancer cells to anti-P-glycoprotein antibody-dependent cytotoxicity of monocytes, but not of lymphocytes. *Jpn. J. Cancer Res.,* **85,** 194–203 (1994).
31) Haku, T., Sone, S., Nabioullin, R. and Ogura, T. Human alveolar macrophages augment natural killer cell stimulatory factor (interleukin-12)-inducible killer activity from autologous blood lymphocytes. *Jpn. J. Cancer Res.,* **86,** 81–87 (1995).
32) Sone, S., Orino, E., Mizuno, K., Yano, S., Nishioka, Y., Haku, T., Nii, A. and Ogura, T. Production of IL-1 and its receptor antagonist is regulated differently by IFN-γ and IL-4 in human monocytes and alveolar macrophages. *Eur. Respir. J.,* **7,** 657–663 (1994).
33) Miyake, M., Ito, M., Hitomi, S., Ikeda, S., Taki, T., Kurata, M., Hino, A., Miyake, N. and Kannagi, R. Generation of two murine monoclonal antibodies that can discriminate *N*-glycolyl and *N*-acetyl neuraminic acid residues of GM_2 gangliosides. *Cancer Res.,* **48,** 6154–6160 (1988).
34) Tripathi, A. K., Taplits, M., Puri, J. and Hoffman, T. Down-regulation of surface FcRI and decrease in antibody-dependent cellular cytotoxicity of cultured monocytes. Reversal by monensin or cytochalasin-D. *J. Immunol.,* **146,** 1309–1315 (1991).
35) Young, D. A., Lowe, L. D. and Clark, S. C. Comparison of the effects of IL-3, granulocyte-macrophage colony-stimulating factor, and macrophage colony-stimulating factor in supporting monocyte differentiation in culture. Analysis of macrophage antibody-dependent cellular cytotoxicity. *J. Immunol.,* **145,** 607–615 (1990).
36) Ellis, T. M., McKenzie, R. S., Simms, P. E., Helfrich, B. A. and Fisher, R. I. Induction of human lymphokine-activated killer cells by IFN-α and IFN-γ. *J. Immunol.,* **143,** 4282–4286 (1989).
37) DeBlaker, H. D. F., Yamauchi, A., Yu, C. R., Horvath, A. J. A. and Bloom, E. T. IL-12 synergizes with IL-2 to induce lymphokine-activated cytotoxicity and perforin and granzyme gene expression in fresh human NK cells. *Cell. Immunol.,* **165,** 33–43 (1995).
38) Mueller, B. M., Romerdahl, C. A., Gillies, S. D. and Reisfeld, R. A. Enhancement of antibody-dependent cytotoxicity with chimeric anti-GD_2 antibody. *J. Immunol.,* **144,** 1382–1386 (1990).
39) Saleh, M. N., Khazaeli, M. B., Wheeler, R. H., Allen, L., Tilden, A. B., Grizzle, W., Reisfeld, R. A., Yu, A. L., Gillies, S. D. and LoBuglio, A. F. A phase I trial of the chimeric anti-GD_2 monoclonal antibody ch14.18 in patients with malignant melanoma. *Hum. Antibodies Hybridomas,* **3,** 19–24 (1992).