Jpn. J. Cancer Res. 85, 298–305, March 1994

Development and Characterization of Chimeric Anti-carcinoembryonic Antigen Monoclonal Antibodies and Their Fab Fragments

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In an attempt to reduce the immunogenicity of two different murine anti-carcinoembryonic antigen (CEA) monoclonal antibodies (MAbs), KM10 and A10, we produced recombinant mouse/human chimeric MAbs and the respective Fab fragments carrying the variable regions of the murine MAbs. Chimeric A10 Fab fragment was expressed in *Escherichia coli*, and produced in large quantities in a mini-jar fermentation system. In competitive binding assays, chimeric MAbs and their Fab fragments showed identical specificity to human CEA epitopes, as compared to the parental MAbs or Fab fragments. Both chimeric Fab fragments exhibited strong immunohistochemical reactivity with various gastrointestinal carcinomas and no reactivity with CEA-related antigens, such as NCA (nonspecific cross-reacting antigen) or BGPI (biliary glycoprotein I). Furthermore, chimeric KM10 MAb elicited substantially higher antibody-dependent cellular cytotoxicity than the murine MAb. Complement-dependent cytotoxicity *in vitro* was much weaker with chimeric KM10 MAb. These results indicate that chimeric MAbs or Fab fragments could potentially replace the parental murine antibodies or their Fab fragments in therapy or diagnosis of human gastrointestinal carcinomas.

Key words: Chimeric monoclonal antibody — Chimeric Fab fragment — KM10 — A10 — Carcinoembryonic antigen

Monoclonal antibodies (MAbs) and their fragments reactive with tumor-associated antigens are being employed for the diagnosis and therapy of human malignancies.[1-3] We previously produced two murine anti-carcinoembryonic antigen (CEA) MAbs (IgG_1s), KM10 and A10, which recognize different epitopes on human CEA.[4-6] In immuno-histochemical studies, these MAbs showed specific reactivity to carcinomas of the stomach, colon, ampulla of Vater and pancreas. In nude mice bearing antigen-positive gastric cancer xenografts, KM10-adriamycin (ADM) conjugates had more potent anti-tumor activity than free ADM.[4] These reports strongly suggested that KM10 and A10 could be useful MAbs for the therapy and diagnosis of gastrointestinal carcinomas. However, administration of murine MAbs to humans elicited anti-mouse immune responses after frequent injections of murine MAbs.[7-9] We attempted to reduce the immunogenicity of our murine MAbs by two different approaches; 1) preparation of Fab fragment[3] and 2) preparation of mouse/human chimeric MAbs and the respective Fab fragments having the variable regions of the parental murine anti-CEA MAbs. The present report describes our preliminary results on the expression and characterization of chimeric anti-CEA MAbs and their Fab fragments.

MATERIALS AND METHODS

Expression of chimeric antibodies and Fab fragments The chimeric MAbs of A10 and KM10 were constructed with human IgG_1 constant regions and the variable regions of the parental murine MAbs. These chimeric MAbs were expressed from murine myeloma cells (Sp2/0) transfected with various plasmids. Chimeric A10 and KM10 Fab fragments were expressed in *Escherichia coli* and yeast cells, respectively. Two chimeric MAbs and KM10 Fab fragment were prepared and purified at XOMA Corp. Chimeric A10 Fab fragment was expressed in an *E. coli* clone, E101/pING3204, and purified from the culture supernatants of a mini-jar fermentation system. The plasmid pING3204 was derived from pIT106,[10] in which κ and Fd genes were placed under the control of the *Salmonella typhimurium araB* promoter.[11] Therefore, supplementation of L-arabinose induced *E. coli* to express several proteins including the chimeric Fab fragment. The concentration of chimeric A10 Fab fragment secreted from *E. coli* in mini-jar fermentation was measured by enzyme-linked immunosorbent assay

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(ELISA). Goat anti-human gamma-chain antibody (TAGO, Inc.) diluted in phosphate-buffered saline (PBS) was added to a 96-well plate and incubated with 1% bovine serum albumin (BSA)/PBS at 4°C overnight. The plate was washed with PBS, and 50 μl of the culture supernatant diluted with 1% BSA/PBS was added to each well. After a 2 h incubation at 25°C, 1000-fold-diluted horseradish peroxidase-conjugated goat anti-human kappa-chain antibody (TAGO) with 1% BSA/PBS was added. Chimeric Fab fragment, which recognizes a different epitope from KM10 or A10, was purchased from XOMA Corp. and utilized as a standard chimeric Fab fragment in ELISA. Each well was washed with PBS and then exposed to substrate solution containing 0.015% H_2O_2 and 2.8 m*M* *o*-phenylenediamine (OPD) in 0.1 *M* phosphate citrate buffer (pH 5.0). After 15 min, 50 μl of 4 *M* H_2SO_2 was added to each well and the absorbance at 490 nm was measured with an auto-reader (Micro-plate reader M-Tmax, Wako Ind., Ltd.).

Purification of chimeric A10 Fab fragment Chimeric A10 Fab fragment was purified from *E. coli* fermentation supernatants using ion-exchange chromatography. Culture supernatant was filtered through a 300 kd exclusion filter (membrane type Omega, Filtron Technology Corp.). The filtrate was concentrated and passed through a 10 kd exclusion filter (Filtron). The concentrated Fab fragment was purified by passing it through a CM Sepharose ion-exchange column at pH 5.1. The chimeric Fab fragment was mostly eluted from the column with 0.1 *M* NaCl. Subsequently, the eluted material was loaded onto a phenyl Sepharose column with 10 m*M* sodium phosphate buffer and 1 *M* $(NH_4)_2SO_4$ (pH 6.5). The chimeric Fab fragment was eluted with 10 m*M* sodium phosphate buffer (pH 6.5) and dialyzed with 10 m*M* sodium phosphate buffer, 0.3 *M* NaCl and 0.1% NaN_3 (pH 6.7). The purity of chimeric MAbs and Fab fragments was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (4–20%) in comparison with parental murine MAbs and Fab fragments. Murine KM10 and A10 MAbs were produced by standard hybridoma technology and purified as described previously.[4–6] Both murine Fab fragments were obtained from parental murine MAbs by papain digestion.

Competitive binding inhibition assay To compare immunoreactivity of chimeric antibodies and their fragments to the parental murine MAbs and their Fab fragments, we used a competitive binding inhibition assay with probes of biotinylated murine KM10 Fab fragment or murine A10 MAb. Biotinylated KM10 Fab fragment or A10 antibody were prepared by mixing murine MAb or Fab fragment (1 mg/ml in 0.1 *M* $NaHCO_3$) and NHS-AHLC-biotin (N-hydroxysuccinimide-aminohexyl long chain-biotin) (10 mg/ml in N,N-dimethylformamide) at 25°C for 3 h. The wells of flat-bottomed 96-well polyvinyl chloride microtiter plates were coated with human CEA in 50 μl of PBS. After overnight incubation at 4°C, the plate was washed with PBS and incubated overnight at 4°C after addition of varying amounts of chimeric KM10 MAb and Fab fragment in 1% BSA/PBS to the wells. The plate was then washed with PBS, and incubated overnight at 4°C with 50 μl of biotinylated murine KM10 Fab fragment (2 μg/ml) in 1% BSA/PBS. Subsequently, 1,000-fold diluted horseradish peroxidase-Avidin D (Vector Co. Ltd.) was added to each well and the plate was incubated for 2 h at 25°C. Each well was washed with PBS and then exposed to substrate solution containing OPD. The bound peroxidase activity was determined at 490 nm. Similarly, competitive binding assays were performed for chimeric A10 MAb and Fab fragment using biotinylated A10 MAb (2.5 μg/ml in 1% BSA/PBS). The results of specific activity for each MAb and Fab fragment are shown as percentage inhibition (% inhibition).

Immuno-histochemical reaction of chimeric Fab fragments The immunoreactivity of chimeric and murine Fab fragments of both KM10 and A10 was tested on cryostat sections of malignant and normal tissues which had been obtained at the time of operation from patients at the First Department of Surgery, Kobe University School of Medicine. These tissues were fixed with 4% paraformaldehyde in 0.1 *M* phosphate buffer (pH 7.4) and embedded in optimum cutting temperature (OCT) compound (Miles Lab., Inc.). Human peripheral leukocytes were obtained from heparinized blood of healthy adults. Frozen sections were stained by the biotin-avidin method using biotinylated chimeric and murine Fab fragments as described previously.[4] As a negative control, cryostat sections were stained with biotinylated Fab fragment of murine anti-HBs antibody.[12] All biotinylated Fab fragments were produced by the same method as the biotinylated murine KM10 Fab fragment.

Immunoactivity of chimeric MAbs The studies of antibody-dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) were performed for two chimeric MAbs as compared to the parental murine antibodies. Anti-leukocyte horse IgG and human IgG purchased from Green Cross Corp. were utilized as positive and negative control antibody, respectively. In the test of ADCC, human peripheral blood mononuclear cells obtained from healthy donors were used as effector cells and the human gastric cancer cell line, MKN-45 was utilized as target cells. Various amounts of IgGs were mixed with ^{51}Cr-labeled target cells before adding effector cells. Effector cells were added to each well at a fixed E/T (effector cells/target cells) ratio of 250:1 and the plate was incubated for 3–4 h at 37°C. The supernatant was collected from each well and the release of radioactivity was measured. In the CDC, ^{51}Cr-labeled MKN-45

cells were incubated with a mixture of various concentrations of antibodies and human serum as a source of human complement for 3 h at 37°C. Release of radioactivity in the culture supernatants was determined. The percentage cytotoxicity (% cytotoxicity) in both ^{51}Cr-release assays was calculated from the following formula:

$$\%\ \text{cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100.$$

RESULTS

Expression of chimeric MAbs and Fab fragments Chimeric A10 Fab fragment was expressed in an *E. coli* clone, E101/pING3204, in a mini-jar fermentation system. The growth of *E. coli* was inhibited and vigorous lysis was observed upon the addition of L-arabinose. However, the L-arabinose supplement induced *E. coli* to secrete chimeric Fab fragment and was not found to affect chimeric A10 Fab fragment expression. The final concentration of chimeric Fab was about 220 mg/liter in culture supernatants as measured by ELISA (Fig. 1). The culture supernatants were found to contain free κ chain, detected by peroxidase-labeled goat anti-kappa chain antibody (TAGO) as described previously[10] (data not shown).

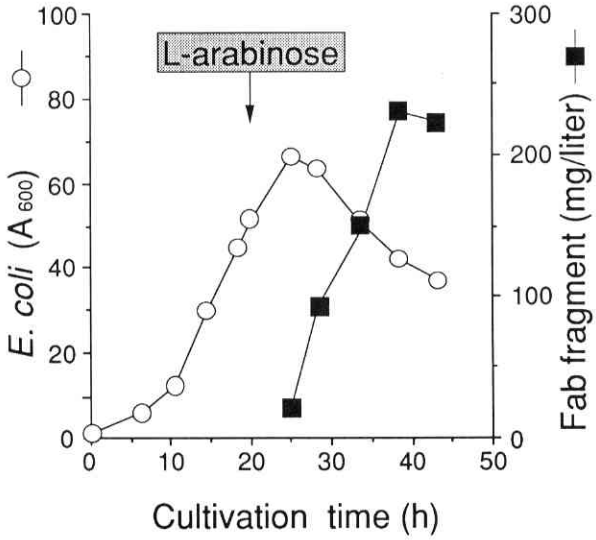

Fig. 1. The production of chimeric A10 Fab fragment and the growth of *E. coli* in a mini-jar fermentation system. The concentration of chimeric Fab fragment (■) secreted from *E. coli* in the culture supernatants was measured by sandwich ELISA and the *E. coli* growth (○) was measured in terms of absorbance at 600 nm. The L-arabinose supplement induced the *E. coli* to express chimeric Fab fragment, while the *E. coli* growth was inhibited and vigorous lysis was observed.

SDS-PAGE analysis SDS-PAGE analyses followed by Coomassie blue staining indicated the essential purity of each preparation of chimeric MAbs and Fab fragments. Under reducing gel conditions, both chimeric MAbs gave 23.5 kd and 50 kd bands (Fig. 2). Purified chimeric KM10 and A10 Fab fragments showed bands of slightly higher molecular size (23.5 kd) than the murine and human Fab fragments (Fig. 3). Under non-reducing gel conditions, chimeric MAbs and Fab fragments exhibited higher molecular sizes than native murine antibodies and their fragments. The differences of molecular sizes may reflect a minor change of amino acid sequence at the V-D-J region of the molecules (Figs. 2 and 3).

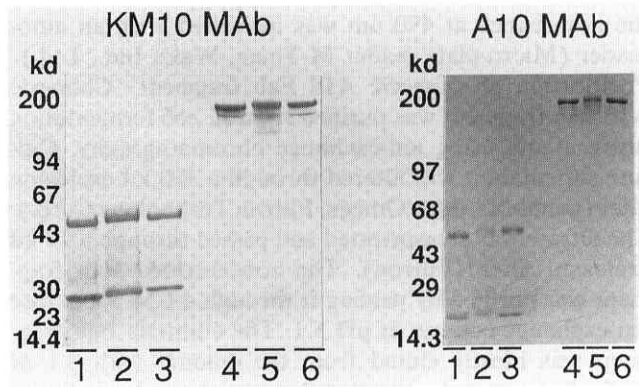

Fig. 2. SDS-PAGE analyses of chimeric KM10 and A10 MAbs. Lanes 1 and 4; murine MAbs, lanes 2 and 5; human polyclonal MAbs and lanes 3 and 6; chimeric MAbs under reducing conditions (lanes 1–3) or non-reducing conditions (lanes 4–6).

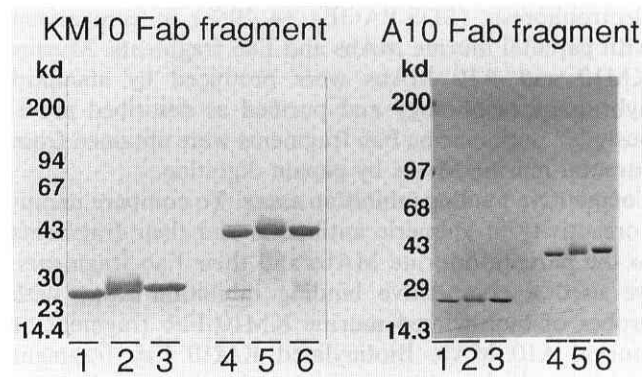

Fig. 3. SDS-PAGE analyses of chimeric KM10 and A10 Fab fragments. Lanes 1 and 4; murine Fab fragments, lanes 2 and 5; human polyclonal Fab fragments and lanes 3 and 6; chimeric Fab fragments under reducing conditions (lanes 1–3) or non-reducing conditions (lanes 4–6).

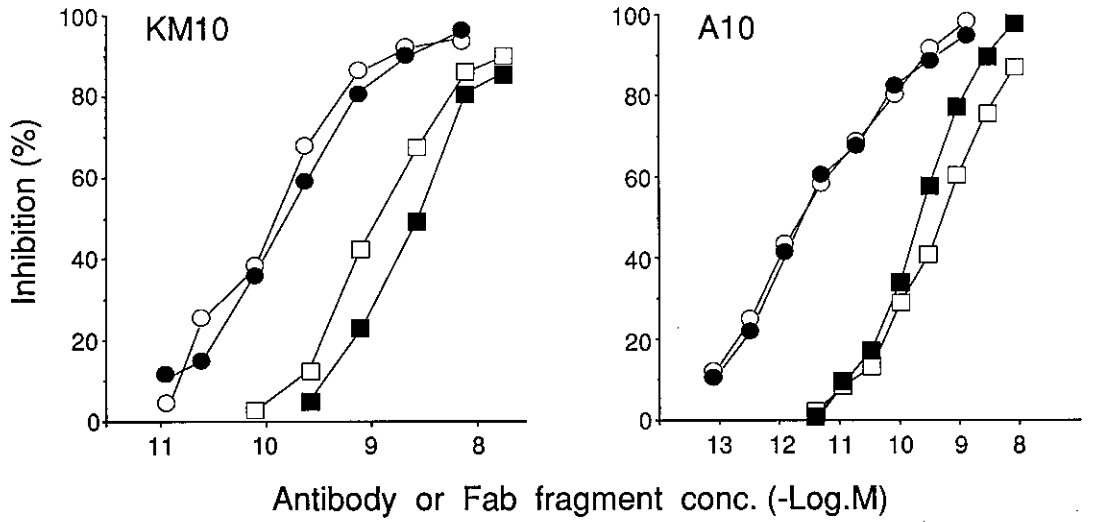

Fig. 4. Immunoreactivity of chimeric MAbs and Fab fragments to human CEA. The affinity of chimeric KM10 and A10 MAbs (●) or Fab fragments (■) were tested in competitive binding assays, as compared to native murine MAbs (○) or Fab fragments (□). The epitope specificity of chimeric MAbs or Fab fragments was equivalent to that of native murine MAbs or Fab fragments.

Immunoreactivity of chimeric MAbs and Fab fragments to human CEA Specific affinity of each MAb or Fab fragment to human CEA was tested by competitive binding inhibition assays with biotinylated parental MAb or Fab fragment. Our results demonstrated that chimeric MAb and Fab fragment had specific immunoreactivity identical with that of their respective parental murine MAb and Fab fragment. While there were no notable differences between chimeric and murine MAbs or Fab fragments in terms of avidity, greater protein inputs of Fab fragments were needed to obtain the same competition levels as those obtained with the MAbs, regardless of whether the Fab fragments were native or chimeric (Fig. 4).

Immuno-histochemical reactivity of chimeric Fab fragments The reactivity of chimeric KM10 and A10 Fab fragments with various malignant and normal tissues was examined by the biotin-avidin method. In the specimens of malignant tumors, all 8 cases (2 cases of gastric cancer, 2 cases of colon cancer, 3 cases of pancreatic carcinoma and 1 case of gallbladder carcinoma) showed positive reactions with both chimeric and murine Fab fragments. Chimeric KM10 Fab fragment demonstrated strongly positive staining at the luminal side and cytoplasmic staining in the well differentiated pancreatic adenocarcinoma. Similarly, chimeric A10 Fab fragment showed a luminal staining pattern in moderately differentiated pancreatic adenocarcinoma. Differences in the positive findings between the two chimeric Fab fragments were found in the degree of biotinylation with their frag-

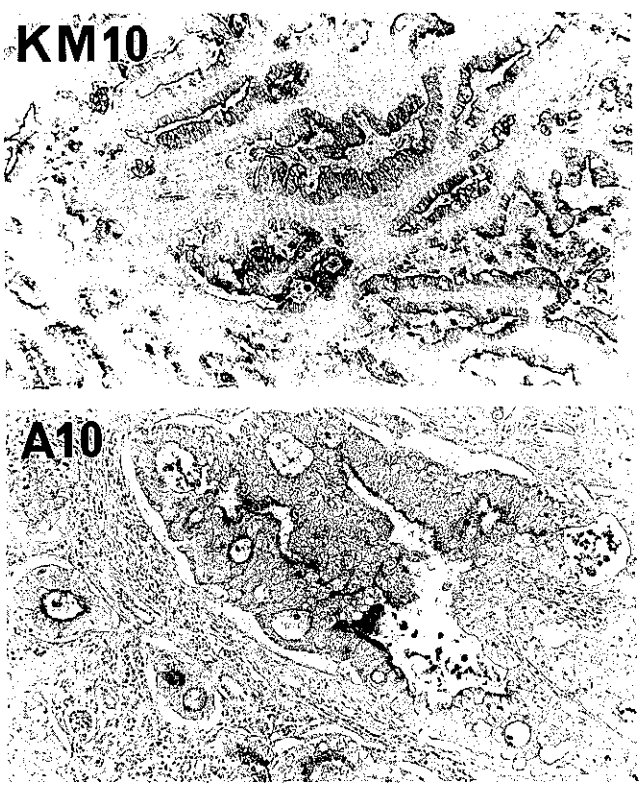

Fig. 5. Immuno-histochemical reaction of chimeric KM10 and A10 Fab fragments to pancreatic carcinomas. Chimeric Fab fragments of KM10 and A10 had specific reactivity with pancreatic carcinomas. The two specimens were obtained from different patients. ×90.

Table I. Immuno-histochemical Reactivity of Chimeric KM10 and A10 Fab Fragments with Various Malignant and Normal Tissues

	Chimeric Fab Fragment		Murine Fab fragment		
	KM10	A10	KM10	A10	anti-HBs
Carcinoma					
pancreas	++	++	++	++	−
stomach	++	++	++	++	−
colon	++	++	++	++	−
gallbladder	++	++	++	++	−
Normal tissue					
stomach	+	+	+	+	−
colon	+	+	+	+	−
bile duct	−	−	−	−	−
granulocyte	−	−	−	−	−

++: strongly positive, +: weakly positive, −: negative.

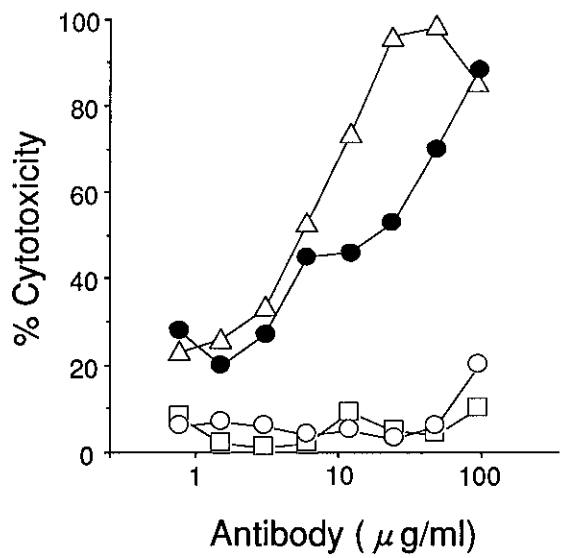

Fig. 6. Antibody-dependent cellular cytotoxicity (ADCC) with chimeric KM10 MAb. Effector cells were normal human peripheral blood mononuclear cells and ^{51}Cr-labeled targets were MKN-45, gastric cancer cells. Chimeric KM10 MAb (●) elicited substantial ADCC activity similar to that induced by horse anti-leukocyte IgG (△). Murine KM10 MAb (○) or human IgGs (□) exhibited no ADCC activity against MKN-45 cells.

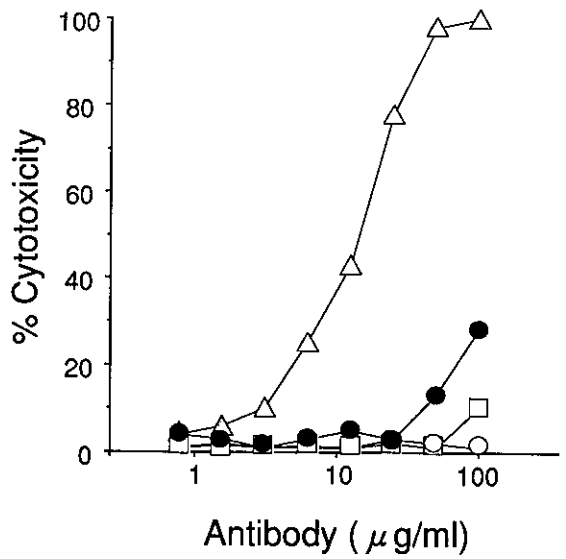

Fig. 7. Complement-dependent cytotoxicity (CDC) with chimeric KM10 MAb. Chimeric KM10 MAb (●) exhibited weak CDC activity, while the positive control anti-leukocyte IgG (△) showed strong CDC activity. Murine KM10 MAb (○) elicited no CDC activity, being similar to human IgGs (□).

ments (Fig. 5). The malignant tissues did not stain with a biotinylated Fab fragment of anti-HBs antibody which was used as the negative control. In normal tissues, chimeric Fab fragments reacted weakly with stomach and colon. The reactions were apical, localizing as a thin line along the luminal side of the mucosal cells. Granulocyte and liver bile duct, which express antigens associated with CEA, were not stained with chimeric Fab fragments, or with murine Fab fragments (Table I).

ADCC and CDC of chimeric MAbs *In vitro* reactivity in ^{51}Cr-release assays of chimeric MAbs and native murine antibodies was tested with antigen-positive human gastric cancer cells (MKN-45) as target cells and normal peripheral blood mononuclear cells as effector cells. Chimeric KM10 MAb mediated significant ADCC at the E/T ratio of 250:1, similar to anti-leukocyte horse IgG which was used as a positive control. There was no notable enhancement of ADCC in the presence of parental murine KM10 MAb (Fig. 6). In the study of CDC, chimeric KM10 antibody was able to elicit only cytotoxicity (only 26.5% of target cells were lysed at the maximal concentration of 100 μg/ml), while no cytotoxicity was seen with murine MAb (Fig. 7). On the other hand, chimeric A10 MAb did not mediate ADCC or CDC, being similar to the parental murine MAb (data not shown).

DISCUSSION

In an attempt to reduce the immunogenicity of monoclonal antibodies for cancer therapy or diagnosis in humans, we have developed a human MAb (IgM) with specific reactivity to human gastrointestinal cancers.[13] However, IgM MAb may not be suitable as a therapeutic or diagnostic agent for human malignancies because of its large molecular size. An alternative approach is to

develop chimeric MAbs using gene engineering technology to reduce immunogenicity. The production of genetically engineered human/mouse chimeric MAbs or their fragments using variable regions from native murine MAbs has been reported.[14-17] Recently, several investigators have shown that fully functional fragments can be expressed directly from bacteria such as *E. coli* or from yeast cells, eliminating the need for proteolytic digestion.[10, 18, 19] In this study, we have demonstrated the direct expression of chimeric mouse/human anti-CEA MAbs and their Fab fragments and the large-scale production of a chimeric A10 Fab fragment in mini-fermentation jars. The ability to produce large amounts of chimeric Fab fragment will enable administration of larger doses of this MAb fragment, which may improve its efficacy. We demonstrated that the chimeric MAbs and Fab fragments produced in large quantities were very pure and retained their specific activity against human CEA proteins. The presence of CEA-related antigens in normal tissues is one of the major problems for the clinical use of anti-CEA MAbs in humans. CEA-related antigens were reported to be present in granulocytes (nonspecific cross-reacting antigen: NCA)[20, 21] and bile canaliculi in the liver (biliary glycoprotein I: BGPI).[22, 23] Nap *et al.* reported that at least 33 antibodies of 52 well-characterized anti-CEA MAbs purchased from 12 different research groups cross-reacted with granulocytes and some of these antibodies stained bile canaliculi or ducts in the liver, while all MAbs reacted with mucosa of the stomach or colon.[24] In the present studies, chimeric Fab fragments reacted weakly with normal stomach or colon. However, our chimeric Fab fragments of anti-CEA MAbs had specific reactivity with gastrointestinal cancers and exhibited no reactivity with granulocytes or liver bile duct in the immuno-histochemical studies. We have previously reported the same pattern of reactivity with the parental murine MAbs.[6] Immuno-histochemical reaction of chimeric MAbs with human malignant tissues was not studied here, but these antibodies had identical epitope specificity with the Fab fragments.

Chimeric mouse/human IgG_1 MAbs were reported to mediate significant ADCC by human effector cells.[25, 26] Our present study demonstrated that chimeric KM10 MAb had substantial ADCC activity and only moderate CDC activity, while specific ADCC or CDC for parental murine MAb was not found. However, high uptake of chimeric MAb in tumors will be needed for therapy of gastrointestinal carcinomas in humans, since the concentration of chimeric KM10 MAb required to elicit sufficient ADCC was high. Therefore, intra-tumoral or selective arterial injection with chimeric KM10 MAb could be useful to obtain greater ADCC than is possible with systemic injection. On the other hand, chimeric A10 MAb did not mediate ADCC or CDC. We assume that chimeric Fab fragments would not mediate ADCC or CDC with human effector cells or human complement, because these fragments lack the Fc portion. However, the removal of the Fc segment could reduce the non-specific binding to Fc receptors found in the reticulo-endothelial system, such as liver, spleen and lung. In biodistribution studies of animal models bearing tumor xenografts, radiolabeled murine Fab fragments afforded a clearer and more rapid detection of tumors as compared to radiolabeled murine whole IgGs.[27, 28] Thus, chimeric Fab fragments may be better candidates for tumor-localization studies than chimeric or murine MAbs.

The presence of the murine native variable regions in these chimeric anti-CEA MAbs and Fab fragments may still elicit anti-idiotype antibody response in humans. LoBuglio *et al.* demonstrated that one of ten patients who received chimeric MAb developed anti-idiotype response.[29] However, the anti-idiotype antibody developed by injection of chimeric MAb or Fab fragment may not react with the other chimeric MAbs or Fab fragments which have different epitope specificity. Therefore, the anti-idiotype antibody immune response may be reduced by using chimeric KM10 and A10 MAbs or Fab fragments, since KM10 and A10 have different epitope specificity.

In the future, biodistribution and anti-tumor activities of these chimeric MAbs and Fab fragments will be explored in animal models bearing antigen-positive tumor xenografts. Based on such studies, clinical trials will be designed to determine which radiolabeled MAbs or Fab fragments are the most useful diagnostic agents and which combinations of native chimeric KM10 MAb and conjugated antibodies or fragments with chemotherapeutic drugs, toxins or radionuclides can be administered for the treatment of various gastrointestinal carcinomas.

ACKNOWLEDGMENTS

The authors wish to thank Dr. William Harel (Alpha Therapeutic Corp., Los Angeles, USA) for his helpful comments and a critical review of the manuscript. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture and for Cancer Research from the Ministry of Health and Welfare, Japan.

(Received August 19, 1993/Accepted December 10, 1993)

REFERENCES

1) Mach, J. P., Buchegger, F., Forni, M., Ritschard, J., Berche, C., Lumbroso, J. D., Schreyer, M., Girardet, C., Accola, R. S. and Carrel, S. Use of radiolabeled monoclonal anti-CEA antibodies for the detection of human carcinomas by external photoscanning and tomoscintigraphy. *Immunol. Today,* **2,** 239–249 (1981).
2) Dillman, R. O., Shawler, D. L., Sobol, R. E., Collins, H. A., Beauregard, J. C., Wormsley, S. B. and Royston, I. Murine monoclonal antibody therapy in two patients with chronic lymphocytic leukemia. *Blood,* **59,** 1036–1045 (1982).
3) Larson, S. M., Carrasquillo, J. A., Krohn, K. A., Brown, J. P., Guffin, R. W., Ferens, J. M., Graham, M. M., Hill, L. D., Beaumier, P. L. and Hellstrom, K. E. Localization of ^{131}I-labeled p97-specific Fab fragments in human melanoma as a basis for radiotherapy. *J. Clin. Invest.,* **72,** 2101–2114 (1983).
4) Ohyanagi, H., Ishida, H., Ishida, T., Soyama, N., Yamamoto, M., Okumura, S., Kano, Y., Ueda, Y. and Saitoh, Y. A monoclonal antibody KM10 reactive with human gastrointestinal cancer and its application for immunotherapy. *Jpn. J. Cancer Res.,* **79,** 1349–1358 (1988).
5) Ishida, H., Ohyanagi, H., Okumura, S. and Saitoh, Y. A monoclonal antibody KM10 reactive with human gastrointestinal cancer. *J. Jpn. Surg. Soc.,* **89,** 508–515 (1988) (in Japanese).
6) Soyama, N., Yamamoto, M., Ohyanagi, H. and Saitoh, Y. Comparative studies on monoclonal antibody KM10 and anti-CEA monoclonal antibodies. *J. Jpn. Surg. Soc.,* **90,** 1834–1839 (1989) (in Japanese).
7) Schroff, R. W., Foon, K. A., Beatty, S. M., Oldham, R. K. and Morgan, A. C., Jr. Human anti-murine immunoglobulin response in patients receiving monoclonal antibody therapy. *Cancer Res.,* **45,** 879–885 (1985).
8) Shawler, D. L., Bartholomew, R. M., Smith, L. M. and Dillman, R. O. Human immune response to multiple injections of murine monoclonal IgG_1. *J. Immunol.,* **135,** 1530–1535 (1985).
9) Courtency-Luck, N. S., Epenetos, A. A., Moore, R., Larche, M., Pectasides, D., Dhokia, B. and Ritter, M. A. Development of primary and secondary immune response to mouse monoclonal antibodies used in the diagnosis and therapy of malignant neoplasms. *Cancer Res.,* **46,** 6489–6493 (1986).
10) Better, M., Chang, C. P., Robinson, R. R. and Horwitz, A. H. *Escherichia coli* secretion of an active chimeric antibody fragment. *Science,* **240,** 1041–1043 (1988).
11) Johnston, S., Lee, J, H. and Ray, D. S. High-level expression of M13 gene II protein from an inducible polycistronic messenger RNA. *Gene,* **34,** 137–145 (1985).
12) Uemura, Y., Fukuyama, K., Nishida, M., Suyama, Y. and Ohori, H. Establishment of passive hemagglutination assay (PHA) system for anti-HBc in plasma. *Tohoku J. Exp. Med.,* **149,** 11–20 (1986).
13) Soyama, N., Ohyanagi, H. and Saitoh, Y. Production of human monoclonal antibody reactive with gastrointestinal carcinoma. *Jpn. J. Gastroenterol.,* **87,** 2635–2641 (1990) (in Japanese).
14) Morrison, S., Johnson, M. J., Herzenberg, L. A. and Oi, V. T. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. Natl. Acad. Sci. USA,* **81,** 6851–6855 (1984).
15) Boulianne, G. L., Hozumi, N. and Shulman, M. J. Production of functional chimeric mouse/human antibody. *Nature,* **312,** 643–646 (1984).
16) Buchegger, F., Pelegrin, A., Hardman, N., Heusser, C., Lukas, J., Dolci, W. and Mach, J. P. Different behaviour of mouse-human chimeric antibody $F(ab')_2$ fragments of IgG_1, IgG_2 and IgG_4 sub-class *in vivo*. *Int. J. Cancer,* **50,** 416–422 (1992).
17) Siegall, C. B., Gawlak, S. L., Chin, J. J., Zoeckler, M. E., Kadow, K. F., Brown, J. P. and Braslawsky, G. R. Cytotoxicity of chimeric (human-mouse) monoclonal antibody BR96 IgG, $F(ab')_2$ and Fab′ conjugated to *Pseudomonas* exotoxin. *Bioconjugate Chem.,* **3,** 302–307 (1992).
18) Skerra, A. and Plückthun, A. Assembly of a functional immunoglobulin Fv fragment in *Escherichia coli. Science,* **240,** 1038–1041 (1988).
19) Horwitz, A. H., Chang, C. P., Better, M., Hellstrom, K. E. and Robinson, R. R. Secretion of functional antibody and Fab fragment from yeast cells. *Proc. Natl. Acad. Sci. USA,* **85,** 8678–8682 (1988).
20) von Kleist, S., Chavanel, G. and Burtin, P. Identification of an antigen from normal human tissue that crossreacts with the carcinoembryonic antigen. *Proc. Natl. Acad. Sci. USA,* **69,** 2492–2494 (1972).
21) Bordes, M., Knobel, S. and Martin, F. Carcinoembryonic antigen and related antigens in blood cells and hematopoietic tissues. *Eur. J. Cancer,* **11,** 783–786 (1975).
22) Svenberg, T. Carcinoembryonic antigen-like substances of human bile. Isolation and partial characterization. *Int. J. Cancer,* **17,** 588–596 (1976).
23) Svenberg, T., Hammarström, S. and Hedin, A. Purification and properties of biliary glycoprotein (BGPI). Immunochemical relationship to carcinoembryonic antigen. *Mol. Immunol.,* **16,** 245–252 (1979).
24) Nap, M., Hammarström, M. L., Bormer, O., Hammarström, S., Wagener, C., Handt, S., Schreyer, M., Mach, J. P., Buchegger, F., von Kleist, S., Grunert, F., Seguin, P., Fuks, A., Holm, R. and Lamerz, R. Specificity and affinity of monoclonal antibodies against carcinoembryonic antigen. *Cancer Res.,* **15,** 2329–2339 (1992).
25) Brüggemann, M., Williams, G. T., Bindon, C. I., Clark, M. R., Walker, M. R., Jeffris, R., Waldmann, H. and Neuberger, M. S. Comparison of the effector function of human immunoglobulins using a matched set of chimeric antibodies. *J. Exp. Med.,* **166,** 1351–1361 (1987).

26) Steplewski, Z., Sun, L. E., Shearman, C. W., Ghrayeb, J., Daddona, P. and Koprowski, H. Biological activity of human-mouse IgG1, IgG2, IgG3, and IgG4 chimeric monoclonal antibodies with antitumor specificity. *Proc. Natl. Acad. Sci. USA,* **85,** 4852–4856 (1988).
27) Wilbanks, T., Peterson, J. A., Miller, S., Kaufman, L., Ortendahl, D. and Ceriani, R. L. Localization of mammary tumors *in vivo* with ^{131}I-labeled Fab fragment of antibodies against mouse mammary epithelial (MME) antigens. *Cancer,* **48,** 1768–1775 (1981).
28) Beaumier, P. L., Krohn, K. A., Carrasquillo, J. A., Eary, J., Hellström, I., Hellström, K. E., Nelp, W. B. and Larson, S. M. Melanoma localization in nude mice with monoclonal Fab against p97. *J. Nucl. Med.,* **26,** 1172–1179 (1985).
29) LoBuglio, A. F., Wheeler, R. H., Trang, J., Haynes, A., Rogers, K., Harvey, E. B., Sun, L., Ghrayeb, J. and Khazaeli, M. B. Mouse/human chimeric monoclonal antibody in man: kinetics and immune response. *Proc. Natl. Acad. Sci. USA,* **86,** 4220–4224 (1989).