Jpn. J. Cancer Res. 85, 172–178, February 1994

Establishment and Characterization of Mouse-Human Chimeric Monoclonal Antibody to *erbB*-2 Product

Tadao Ishida, Masayuki Tsujisaki,[1] Yuji Hinoda, Kohzoh Imai and Akira Yachi

Department of Internal Medicine (Section 1), Sapporo Medical University, S-1, W-16, Chuo-ku, Sapporo 060

A mouse-human chimeric antibody for *erbB*-2 product was established by a new procedure using heavy chain loss mouse mutant hybridoma and human immunoglobulin expression vector. The E401 hybridoma secreted anti-*erbB*-2 product monoclonal antibody (MoAb) (IgG1, κ). The gene of the mouse variable regions of heavy chain was amplified and cloned by the polymerase chain reaction technique directly from the E401 hybridoma RNA. The variable region of heavy chain was joined with the expression vector, which contains human γ1 constant gene. The expression vector was transfected into heavy chain loss mutant cells E401-12, which produced only murine immunoglobulin light chains. A chimeric monoclonal antibody CH401 retained full binding reactivity to *erbB*-2 product, compared with murine E401 parental antibody. Furthermore, the chimeric MoAb CH401 was much more efficient in supporting antibody dependent cell-mediated cytotoxicity activity against *erbB*-2-bearing human adenocarcinoma cells than murine MoAb E401. These suggest that a chimeric monoclonal antibody CH401 may be a potent reagent for therapy of human adenocarcinomas.

Key words: Oncogene — *erbB*-2 product — Chimeric monoclonal antibody

The human c-*erbB*-2 oncogene encodes a 185-kD transmembrane glycoprotein with tyrosine kinase activity.[1)] This *erbB*-2 product has structural and sequence similarity with the epidermal growth factor (EGF) receptor, suggesting that it is a receptor for a growth factor.[2)] In addition, *erbB*-2 protein is necessary for the maintenance of the malignant phenotype of cells transformed by *erbB*-2 gene.[3)] The *erbB*-2 has been found to be amplified in some human adenocarcinomas of the breast (20–30%) and ovary (26%), and tubular adenocarcinomas of the stomach (40%).[4–6)] Down-modulation of *erbB*-2 product might reduce continued malignancy. Therefore, these products are particularly appropriate targets for antitumor immunotherapy.

Clinical application of murine monoclonal antibodies (MoAbs) for diagnosis and therapy in human cancers has been a very active area of research.[7, 8)] These clinical trials included the use of murine MoAbs alone or conjugated to isotopes, toxins, or drugs. But there are various problems. The major limitation in the utility of murine monoclonal antibodies lies in the human anti-mouse antibody (HAMA) response.[9, 10)] Although the use of human monoclonal antibodies may resolve these problems, human MoAbs available for clinical use are limited because of difficulties in the establishment and maintenance of human hybridoma cell lines. The HAMA response is usually directed against the immunoglobulin constant regions although an appreciable anti-idiotypic response is often detected.[11)] Recent advances in gene technology make possible the exchange of mouse constant region domains with human constant region domains, to construct a mouse-human chimeric antibody.[12–14)] Most of the mouse-human chimeric antibodies have been reported to retain the specific binding activities to the antigens which were recognized by their parental mouse antibodies.[15)] Considering that a major portion of the immunogenicity of the antibody molecules is directed against the constant region, the use of mouse-human chimeric antibodies is expected to reduce or eliminate the problems of murine monoclonal antibodies.[16)] Recently, it was shown that chimeric antibody had a longer circulation time than its mouse MoAb.[17, 18)] In addition, chimeric antibodies mediated human complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC) more efficiently than the mouse antibodies.[19, 20)] We have developed the murine monoclonal antibody E401 against the extracellular domain of *erbB*-2 product, and showed that it can be internalized during incubation with cultured cancer cells that overexpress *erbB*-2 product.

In this report, we amplified and cloned the cDNA of the murine heavy chain V region by the polymerase chain reaction (PCR) technique directly from the E401 hybridoma RNA. We then constructed the expression vector, which contains the mouse immunoglobulin variable regions of heavy chain and the human γ1 constant regions. This expression vector was transfected into heavy chain loss mutant cells obtained from E401 hybridoma. The resultant chimeric antibody showed specific binding affinity to the *erbB*-2 product.

[1] To whom correspondence should be addressed.

MATERIALS AND METHODS

Cell lines The mouse hybridoma cell line E401 (IgG1, κ) recognizes the *erbB*-2 product. E401-12 is a heavy chain loss mutant cell line subcloned from E401 and it produces mouse κ light chain. E401-12 was used for transfection of the chimeric genes. The cultured gastric carcinoma cell line JRST was a generous gift from Dr. A. Terano (Faculty of Medicine, University of Tokyo). All the cell lines used in this study were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 2 m*M* L-glutamine and gentamycin sulfate (25 mg/ml).

Primers and cloning of the E401 heavy chain V region cDNA The oligonucleotides were designed using the database of Kabat *et al.*[21] The 5′ primers were constructed from the information available on the conserved sequences of the leader regions. The 5′ end primer to heavy-chain used in this experiment was 5′CATCGATATGGGATGGAGCTGGATCTTTC3′ containing the *Cla*I site. The 3′ primer was constructed from a consensus sequence within the constant region. The primer to the IgG1 heavy-chain was 5′CGGTACCGGTGTCGACTTACCTGAGGAGACTG3′ containing the *Kpn*I site. Total cellular RNA was isolated from the E401 hybridoma cells by the guanidine isothiocyanate method as described by Davis *et al.*[22] The PCR to amplify a segment of the mRNA was carried out according to the method of Kawasaki.[23]

Nucleotide sequencing The amplified PCR fragments of DNA were ligated into Bluescript (Stratagene, CA), and two cDNA clones were directly sequenced for comparison in order to detect nucleotide misincorporations based on PCR by the dideoxy chain termination method using a Sequenase kit (USB, OH).

Construction of chimeric genes and expression vector The expression vector mpSV2neo-EP1-Cγ1 was originally derived from mpSV2neo-EP1-V-Cγ1 vector (kindly donated by Dr. Hozumi),[24] containing the 0.3 kb immunoglobulin heavy-chain promoter fragment, the 1.5 kb immunoglobulin heavy-chain enhancer fragment, the 3.2 kb human IgG genomic Cγ1 fragment and neo gene as a selection marker. The 0.4 kb E401 heavy-chain V region cDNA fragment was ligated into the *Cla*I and *Kpn*I sites of mpSV2neo-EP1-Cγ1 vector, and the expression vector mpSV2neo-EP1-V_{E401}-Cγ1 was constructed as shown in Fig. 1.

Transfection of chimeric heavy chain immunoglobulin gene The chimeric heavy chain expression vector was transfected into heavy-chain loss mutant E401-12 cells by electroporation.[25] The human immunoglobulin expression vector mpSV2neo-EP1-V_{E401}-Cγ1 was transfected and stable transformants were selected in the presence of G418 at 1.0 mg/ml. After 14 days, the growth supernatants were screened for human γ chain expression and binding reactivity to *erbB*-2 product using the binding radioimmunoassay. The cells with the positive human γ chain expression and positive binding to *erbB*-2 product were subcloned and expanded on a large scale for purification. A clone of the stable transformants containing the chimeric gene was established and termed CH401.

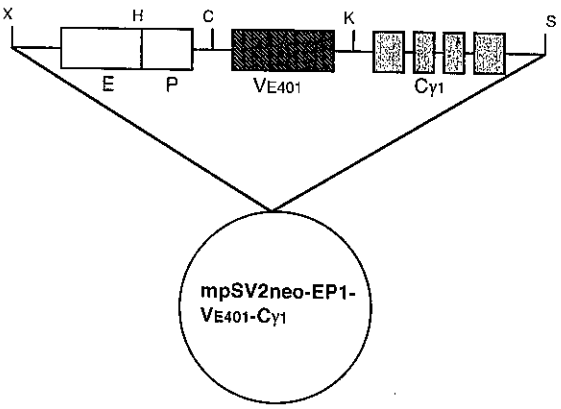

Fig. 1. Schematic diagrams of the construction of the expression vector. The expression vector mpSV2neo-EP1-Cγ1 contains the 0.3 kb murine immunoglobulin heavy-chain promoter fragment (P), the 1.5 kb murine immunoglobulin heavy-chain enhancer fragment (E) and the 3.2 kb human Ig genomic Cγ1 fragment (Cγ1). The expression vector mpSV2neo-EP1-V_{E401}-Cγ1 was constructed by ligating the 0.4 kb E401 heavy-chain V region cDNA fragment into the expression vector mpSV2neo-EP1-Cγ1. Restriction enzymes are abbreviated as follows: *Cla*I, C; *Kpn*I, K; *Xba*I, X; *Hin*dIII, H and *Sal*I, S.

Purification and radiolabeling of monoclonal antibodies The mouse MoAb E401 and chimeric MoAb CH401 from the resulting hybridomas were purified from ascitic fluid either by caprylic acid precipitation,[26] or by affinity chromatography on Protein A-Sepharose.[27] Antibodies were radiolabeled with ^{125}I, utilizing the Chloramine T method.[28]

Binding assays to cultured cells The ^{125}I-labeled MoAb (100 μl, 1×10^5 cpm/well) was incubated with the cultured hybridoma and transfected hybridoma (1×10^5 cells) in multiwell plates for 2 h at 4°C. After 5 washings, the wells were cut out and the radioactivity was counted in a γ-counter. Indirect binding assay was performed as follows: 100 μl of MoAb supernatant was incubated with the cultured gastric carcinoma JRST cells (1×10^5) for 2 h at 4°C. The plate was washed, and 100 μl of ^{125}I-labeled anti-mouse IgG-Fc antibodies or anti-human IgG1-Fc antibodies was added per well and incubated for 2 h at 4°C. After 5 washings, the wells were cut out and the radioactivity was counted in a γ-counter.

Association constants (K_a) of mouse MoAb and chimeric MoAb were measured by means of Scatchard analysis.[29] Briefly, 100 μl aliquots of serially diluted ^{125}I-labeled MoAbs (1.56×10^{-8}–4.87×10^{-10} M) were incubated with *erbB*-2-bearing cells (JRST) for 1.5 h. Following 5 washings, bound radioactivity was counted. B/F and [B] were plotted to obtain K_a from the slope of the line [B, cpm of bound antibodies; F, cpm of free antibody; [B], concentration of bound antibody (M)].

Competition assay In order to test whether the epitopes recognized by murine MoAb E401 and chimeric MoAb CH401 were different, competition assay was performed. Cultured gastric carcinoma JRST cells were preincubated with an excess of unlabeled MoAb for 1 h at 4°C. After washing, ^{125}I-labeled MoAb (1×10^5 cpm/well) was added to test for the ability to bind. After incubation for 1 h, bound radioactivity was counted. Results were calculated as % blocking, compared to the negative control.

ADCC The target cells (JRST) for the ADCC assay were labeled with 100 μCi of ^{51}Cr-sodium chromate (Amersham, Buckinghamshire, UK) for 1 h at 37°C in RPMI-1640 medium. Human peripheral blood mononuclear cells used as effector cells were obtained from a healthy donor and separated by using lymphocyte separation medium (Ficoll-Paque, Sigma). Target cells (1×10^4, 50 μl) and effector cells (100 μl) at the effector/target (E/T) ratio of 25 were added in triplicate to the round-bottomed 96-well microtiter plate with 100 μl of the purified chimeric or mouse monoclonal antibody, and incubated for 8 h at 37°C. After incubation, 100 μl of the supernatant from each well was collected and counted in a γ-counter.

RESULTS

Nucleotide sequences of the V_H region of MoAb E401 The 0.4 kb amplified PCR fragment from the RNA of the E401 hybridoma cells was ligated into Bluescript, and then sequenced by the dideoxy chain termination method. The nucleotide and deduced amino acid sequences of the V_H region of MoAb E401 are shown in Fig. 2. It was assigned to subgroup IIb according to Kabat *et al.*[21] Among members of the same subgroup, the V_H of MoAb 132,16,CL[21] was highly homologous with that of MoAb E401 at the amino acid level. However, the sequences of the CDR2 and CDR3 in the V_H of MoAb E401 were rather specific to MoAb E401 when compared with other members of the subgroup, IIb, including MoAb 132,16,CL, so far reported, suggesting

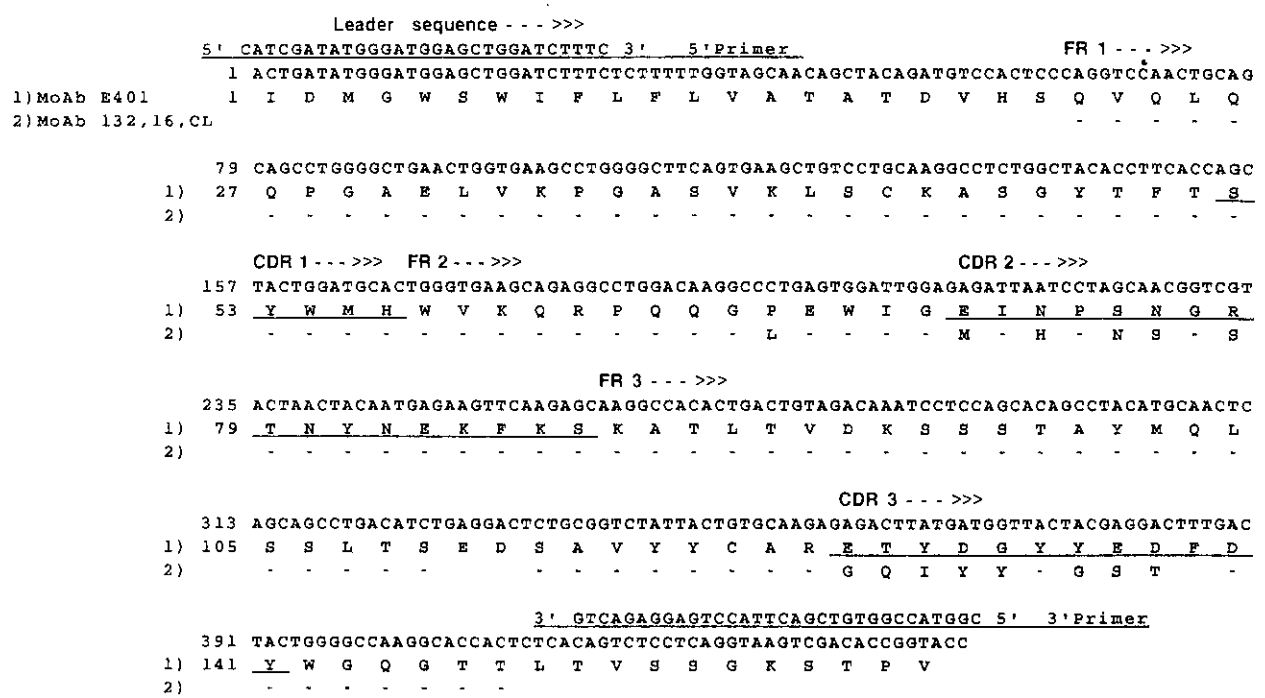

Fig. 2. Nucleotide sequences and deduced amino acids of the V_H cDNA of MoAb E401 and control. Sequences were determined as described in "Materials and Methods." 1), MoAb E401; 2), MoAb 132,16,CL. FR; framework region, CDR; complementary determining region.

that this region could be important for defining the specificity of MoAb E401.

Transfection of chimeric genes and radioimmunoassay The chimeric expression vector was transfected into heavy chain loss mutant cells E401-12 by electroporation. After selection in G418 for 2 weeks, 17 clones were screened for antibody production by radioimmunoassay. The 17 clones were incubated with ^{125}I-labeled anti-mouse IgG-Fc Abs and anti-human IgG-Fc Abs. As shown in Fig. 3, the E401 hybridomas expressed mouse IgG but not human IgG. The heavy-chain loss mutant cells E401-12 expressed neither mouse IgG nor human IgG-Fc. But transfected heavy-chain loss mutant cells CH401-12-1, CH401-12-5 and CH401-12-7 expressed human IgG-Fc. Among the three transfected heavy chain loss mutant cell lines, CH401-12-1 was subcloned and chosen for further analysis.

Specificity and affinity of chimeric antibody In order to test the binding activity of chimeric antibody to the antigen, binding radioimmunoassay was performed using the c-*erbB*-2 gene-transfected SV22 cells or *erbB*-2 product-bearing human gastric adenocarcinoma JRST and MKN7 cells. As shown in Table I, chimeric antibody CH401-12-1 strongly reacted with *erbB*-2-bearing SV22, JRST and MKN7 cells. The affinities of mouse MoAb E401 and chimeric MoAb CH401 were similar since

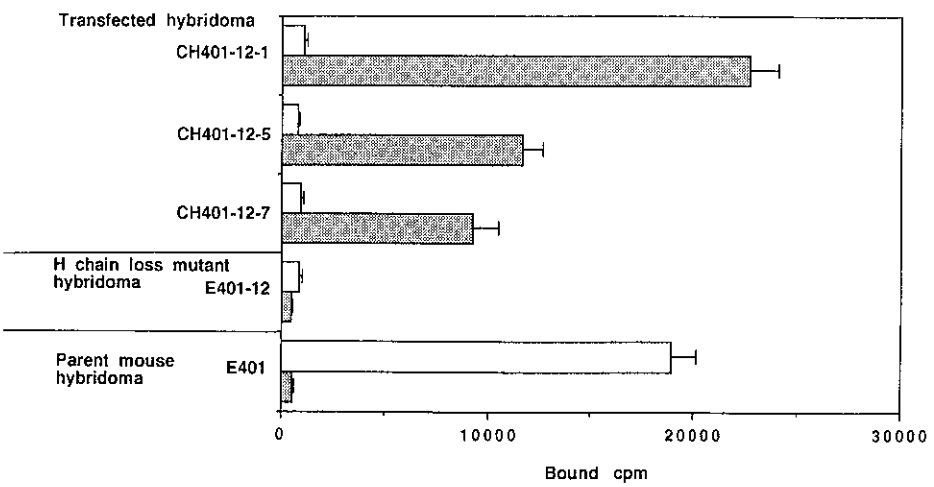

Fig. 3. Expression of human IgG on the cell surface of the hybridomas. The mouse hybridoma (E401), the heavy chain loss mutant hybridoma (E401-12) and the transfected hybridoma (CH401-12-1, CH401-12-5 and CH401-12-7) were tested with ^{125}I-labeled anti-mouse IgG-Fc Ab (□) and anti-human IgG-Fc Ab (▨) in direct binding assay.

Table I. Specificity of Mouse-Human Chimeric Antibody CH401 and Parent Mouse MoAb E401[a)]

Cultured cells[b)]	Mouse-human chimeric antibody CH401-12-1		H-chain loss mutant hybridoma supernatant E401-12		Parent mouse monoclonal antibody E401	
	Anti-mouse IgG Ab	Anti-human IgG Ab	Anti-mouse IgG Ab	Anti-human IgG Ab	Anti-mouse IgG Ab	Anti-human IgG Ab
SV22	1327±94[c)]	16485±766	1350±76	1280±65	24379±823	1244±47
NIH3T3	1230±72	1160±68	1233±83	1142±74	1567±89	1185±73
JRST	1296±60	21356±872	1306±83	1318±92	38015±952	1374±80
MKN7	1338±74	15503±736	1240±75	1393±81	20601±772	1292±83
K562	1158±64	1063±76	1270±81	1107±72	1611±72	1364±75
Molt4	1062±56	1101±61	1297±67	1190±59	1518±81	1201±73

a) Cultured cells were incubated for 2 h at 4°C with supernatant of hybridoma CH401-12-1, E401-12 and E401. After having been washed, the cells were incubated for 2 h with ^{125}I-labeled anti-mouse IgG-Fc antibody or anti-human IgG-Fc antibody. Then the cells were washed and bound radioactivity was counted in a gamma counter.

b) SV22, *erbB*-2-transfected NIH3T3 cells; NIH3T3, mouse fibroblasts; JRST, human gastric cancer cells; MKN7, human gastric cancer cells; K562, human myeloid leukemia cells and Molt4, human T lymphocytic leukemia cells.

c) Results were expressed as bound cpm (values denote mean±SD).

the association constants (K_a) were $2.3\pm0.5\times10^8$ (mean±SD) and $4.0\pm0.4\times10^8$ M^{-1}, respectively.

Competition assay was done to analyze the spatial relationship among epitopes recognized by mouse MoAbs and chimeric MoAb. The epitopes recognized by MoAb E401 and E811 were the same or spatially close, but that recognized by MoAb E919 was different (manuscript in preparation). Table II showed that mouse MoAb E401 and chimeric MoAb CH401-12 inhibited the binding of the other ^{125}I-labeled MoAbs to JRST cells, suggesting that the specificity is the same between these mouse and chimeric MoAbs.

Table II. Epitopes Recognized by Anti-*erbB*-2 Murine Monoclonal Antibodies and Chimeric Antibody

Inhibitor[a]	^{125}I-labeled MoAb			
	E401	E811	E919	CH401-12-1
MoAb E401	95±3[b]	95±4	2±1	93±3
MoAb E811	93±2	94±2	3±1	92±4
MoAb E919	0±1	1±1	96±3	3±1
MoAb CH401-12-1	92±3	93±4	2±1	96±4
E401-12[c]	1±1	0±1	0±1	1±0

a) Human gastric carcinoma JRST cells were incubated with an excess of MoAbs and then tested for the ability to bind ^{125}I-labeled MoAbs.
b) The results are expressed as percentage inhibition (values denote mean±SD).
c) Heavy chain loss mutant E401-12 supernatant as a negative control.

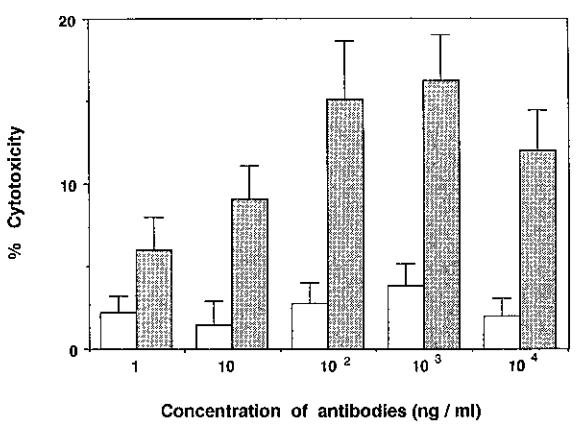

Fig. 4. ADCC activity of chimeric MoAb with human effector cells against JRST cells. Murine MoAb E401 (□) and mouse-human chimeric Ab CH401-12-1 (▨) were used at different concentrations (1, 10, 10^2, 10^3, and 10^4 ng/ml). The E/T ratio was 25. The percentage (mean±SD) cytotoxicity was determined from the results of an 8-h ^{51}Cr-release assay.

ADCC by the chimeric antibody CH401-12 The chimeric antibody possessing the constant region of human origin was tested for ADCC activity using human effector cells. Normal human peripheral blood mononuclear cells were used as effector cells. In ADCC assay JRST cells were used as target cells. As shown in Fig. 4, the chimeric CH401-12 showed stronger cytotoxicity against target cells at the E/T ratio of 25:1 (a maximum of 16.0% versus 3.8%). It is of note that the ADCC activity of the chimeric MoAb CH401-12 at 0.001 μg/ml was almost equal to that of the mouse MoAb E401 at 1 μg/ml. Cytotoxicity was specific to the *erbB*-2 product since ADCC was not observed when *erbB*-2-negative cells were used.

DISCUSSION

The mouse MoAb E401 recognizes an *erbB*-2 product which is expressed on cell surfaces of various human adenocarcinomas. The *in vivo* anti-tumor activity of MoAb E401 has been investigated by using tumor-bearing nude mice. After the administration of MoAb E401, we observed that MoAb E401 was specifically localized on the *erbB*-2 product-bearing SV22 tumor site in nude mice. In addition, it was found that MoAb E401 was internalized into human cancer cells overexpressing *erbB*-2 product during incubation with them. Since this feature of the MoAb is advantageous, we conjugated the MoAb E401 with adriamycin. The conjugated MoAb E401 showed significant cytotoxicity to *erbB*-2-positive JRST cells, but not to cells which did not express *erbB*-2 product. We also found that the incidence of positivity for circulating *erbB*-2 product in the serum of cancer patients was low (approximately 10% of cancer patients in whom cancer cells produced *erbB*-2 product).

Based on these results, *erbB*-2 gene products have the following advantages as a target for cancer therapy: (i) c-*erbB*-2 gene product is a receptor-type molecule expressed on the cell surface, (ii) the expression level in adult normal tissues is very low, (iii) some adenocarcinoma tissues highly express this protein, (iv) the shedding level of this antigen is low, and the incidence of positivity for circulating *erbB*-2 antigen in malignant diseases is about one-tenth of that for cancerous tissues. That is, this antigen is frequently expressed on cancer cells, but not easily released from them. Accordingly, the blocking effect of circulating antigen on the function of MoAb should be negligible, (v) anti-*erbB*-2 MoAb E401 can be internalized into *erbB*-2 product-bearing cancer cells, (vi) this MoAb can be conjugated with anti-tumor agents and has a specific anti-tumor effect against *erbB*-2-bearing cultured cells.

However, the immunogenicity of the mouse MoAb E401 in humans is a disadvantage for its repeated use in

patients, and its functional activity (ADCC and CDC) may be insufficient to effect optimal tumor destruction. It has been estimated that a major immunogenic site resides in the C_{H2} (constant heavy chain 2) region of the IgG molecule.[30] To overcome those problems, we generated chimeric mouse-human MoAb by replacing the heavy chain C domains of the mouse immunoglobulins with those of human immunoglobulins. But the induction of anti-light chain antibody and anti-idiotypic antibody still remains as a problem. We employed the heavy chain V region cDNA fragment, which was amplified and cloned directly from the MoAb E401 hybridoma RNA using the PCR technique. The variable region of heavy chain was joined with the expression vector which contains human γ1 constant gene. The expression vector was transfected into heavy chain loss murine mutant hybridoma cells. As shown in "Results," the chimeric antibody CH401 was found to bind to the *erbB*-2 product on human tumor cells as well as the mouse MoAb E401 (Table I), and the Ch401 obviously recognized the same epitope as MoAb E401 (Table II). Furthermore, the affinity of chimeric antibody CH401 was as high as that of mouse MoAb E401. In this study, we have used a human Cγ1 gene as the constant region of heavy chain, since it was reported that the human Cγ1 element induced an efficient effector function, such as CDC and ADCC, in the human immune system.[19] In fact, our chimeric antibody CH401 was much more efficient than the mouse MoAb E401 in ADCC assay with human effector cells (Fig. 4). The clinical usefulness of chimeric antibodies is limited by the difficulty of producing a large amount of antibody. Further, in some cases, chimeric antibodies showed weaker binding affinity to the antigen. However, these problems have been overcome in the present procedure, since the promoter and enhancer of murine immunoglobulin were introduced into the expression vector, resulting in a higher production of chimeric antibody. In addition the affinities of chimeric MoAb CH401 and mouse MoAb E401 were almost the same. But these chimeric MoAbs can not be used for all cancer patients, because the incidence of *erbB*-2 overexpression in human adenocarcinomas is relatively low (e.g., breast (20–30%), ovarian (25%), and gastric cancer (40%)).

In conclusion, our mouse-human chimeric antibody CH401 specific for *erbB*-2 product is a strong candidate for immunotherapy in patients who are suffering from adenocarcinomas expressing *erbB*-2 product. Moreover, the technology used for the present work should make it possible to construct many other chimeric MoAbs with improved antitumor activity.

ACKNOWLEDGMENTS

We thank Dr. A. Terano (University of Tokyo, Tokyo, Japan) for the gift of cultured JRST cells, and Dr. N. Hozumi (University of Toronto, Toronto, Canada) for the expression vector mpSV2neo-EP1-V-Cγ1. The authors acknowledge the technical help of M. Ohe and secretarial assistance of H. Yamashita. This work was supported by a Grant-in-Aid for Co-operative Research (A.Y., K.I.), a Grant-in-Aid for Scientific Research on Priority Areas (K.I., Y.H.), a Grant-in-Aid for Cancer Research (M.T., K.I., A.Y.) from the Ministry of Education, Science and Culture, and Grants for Cancer Research (A.Y.) from the Ministry of Health and Welfare, Japan.

(Received July 12, 1993/Accepted October 15, 1993)

REFERENCES

1) Akiyama, T., Sudo, C., Ogawara, H., Toyoshima, K. and Yamamoto, T. The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. *Science,* **232,** 1644–1646 (1986).
2) Yamamoto, T., Ikawa, S., Akiyama, T., Semba, K., Nomura, N., Miyajima, N., Saito, T. and Toyoshima, K. Similarity of protein encoded by the human c-erbB-2 gene to epidermal growth factor receptor. *Nature,* **319,** 230–234 (1986).
3) Derbin, J. A., Link, V. C., Stern, D. F., Weinberg, R. A. and Greene, M. I. Down-modulation of an oncogene protein product and reversion of the transformed phenotype by monoclonal antibodies. *Cell,* **41,** 695–706 (1985).
4) Slamon, D. J., Godolphin, W., Jones, L. A., Holt, J. A., Wong, S. G., Keith, D. E., Levin, W. J., Stuart, S. G., Udove, J., Ullrich, A. and Press, M. F. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science,* **244,** 707–712 (1989).
5) Yokota, J., Yamamoto, T., Miyajima, N., Toyoshima, K., Nomura, N., Sakamoto, H., Yoshida, T., Terada, M. and Sugimura, T. Genetic alteration of the c-erbB-2 oncogene occurs frequently in tubular adenocarcinoma of the stomach and often accompanied by amplification of the v-erbA homologue. *Oncogene,* **2,** 283–287 (1988).
6) Venter, D. J., Tuzi, N. L., Kumar, S. and Gullick, W. J. Overexpression of the c-erbB-2 oncoprotein in human breast carcinomas: immunohistological assessment correlates with gene amplification. *Lancet,* **ii,** 69–72 (1987).
7) Houghton, A., Mintzer, D., Cordon-Cardo, C., Welt, S., Fliegel, C., Vadhan, S., Carswell, E., Melamed, M., Oettgen, H. and Old, L. Mouse monoclonal IgG3 antibody detecting GD3 ganglioside: A phase I in patient with malignant melanoma. *Proc. Natl. Acad. Sci. USA,* **82,** 1242–1246 (1985).

8) Colcher, D., Esteban, J. M., Carrasquillo, J. A., Sugarbaker, P., Reynolds, J. C., Bryant, G,. Larson, S. M. and Schlom, J. Quantitative analysis of selective radiolabeled monoclonal antibody localization in metastatic lesions of colorectal cancer patients. *Cancer Res.*, **47**, 1185–1189 (1987).
9) LoBuglio, A. F., Saleh, M. N., Lee, J., Khazaeli, M. B., Carrano, R., Holden, H. and Wheeler, R. H. Phase I trial of multiple large doses of murine monoclonal antibody CO17-1A. I. Clinical aspects. *J. Natl. Cancer Inst.*, **80**, 932–936 (1988).
10) Khazaeli, M. B., Saleh, M. N., Wheeler, R. H., Huster, W.J., Holden, H., Carrano, R. and LoBuglio, A. F. Phase I trial of multiple large doses of murine monoclonal antibody CO17-1A. II. Pharmacokinetics and immune response. *J. Natl. Cancer Inst.*, **80**, 937–942 (1988).
11) Courtenay-Luck, N. S., Epenetos, A. A., Moore, R., Larche, M., Pectasides, D., Dhokia, B. and Ritter, M. A. Development of primary and secondary immune responses to mouse monoclonal antibodies used in the diagnosis and therapy of malignant neoplasms. *Cancer Res.*, **46**, 6489–6493 (1986).
12) Boulianne, G. L., Hozumi, N. and Shulman, M. J. Production of functional chimeric mouse/human antibody. *Nature*, **312**, 643–646 (1984).
13) Morrison, S. L., Johnson, M. J., Herzenberg, L. 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).
14) Riechmann, L., Clark, M., Waldmann, H. and Winter, G. Reshaping human antibodies for therapy. *Nature*, **332**, 323–327 (1988).
15) Sahagan, B. G., Dorai, H., Saltzgaber-Muller, J., Toneguzzo, F., Guindon, C. A., Lilly, S. P., McDonald, K. W., Morrissey, D. V., Stone, B. A., Davis, G. L., McIntosh, P. K. and Moore, G. P. A genetically engineered murine/human chimeric antibody retains specificity for human tumor-associated antigen. *J. Immunol.*, **137**, 1066–1074 (1986).
16) Larson, S. M., Garrasquillo, J. A., Krohn, K. A., Brown, J. P., McGuffin, R. W., Ferens, J. M., Graham, M. M., Hill, L. D., Beaumier, P. L., Hellström, K. E. and Hellström, I. Localization of ^{131}I-labeled p^{97}-specific Fab fragments in human melanoma as a basis for radiotherapy. *J. Clin. Invest.*, **72**, 2101–2114 (1983).
17) LoBuglio, A. F., Wheeler, R. H., Trang, J., Haynes, A., Rogers, K., Hervey, E. B., Sun, L., Ghrayéb, 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).
18) Kahazaeli, M. B., Saleh, M. N., Liu, T. P., Meredith, R. F., Wheeler, R. H., Baker, T. S., King, D., Secher, D., Allen, L., Rogers, K., Colcher, D., Schlom, J., Shochat, D. and LoBuglio, A. F. Pharmacokinetics and immune response of ^{131}I-chimeric mouse/human B72.3 (human γ4) monoclonal antibody in humans. *Cancer Res.*, **51**, 5461–5466 (1991).
19) Liu, A. Y., Robinson, R. R., Hellstrom, K. E., Murray, E. D., Chang, C. P. and Hellström, I. Chimeric mouse-human IgG1 antibody that can mediate lysis of cancer cells. *Proc. Natl. Acad. Sci. USA*, **84**, 3439–3443 (1987).
20) Nishimura, Y., Yokota, M., Akira, K., Ueda, R., Kudo, A. and Watanabe, T. Recombinant human-mouse chimeric monoclonal antibody specific for common acute lymphocytic leukemia antigen. *Cancer Res.*, **47**, 999–1005 (1987).
21) Kabat, E. A., Wu, T. T., Reid-Miller, M., Perry, H. M. and Gottesman, K. S. "Sequences of Proteins of Immunological Interest," 4th Ed. (1987). U.S. Dept. Health and Human Services, Washington, D.C.
22) Davis, L. G., Dibner, M. D. and Battey, J. F. Guanidine isothiocyanate preparation of total RNA. *In* "Basic Methods in Molecular Biology," pp. 91–96 (1986). Elsevier, New York.
23) Kawasaki, E. S. Amplification of RNA. *In* "PCR Protocols. A Guide to Methods and Applications," ed. M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White, pp. 21–27 (1990). Academic Press Inc., San Diego.
24) Xiang, J., Roder, J. and Hozumi, N. Production of murine V-human Cγ1 chimeric anti-TAG72 antibody using V region cDNA amplified by PCR. *Mol. Immunol.*, **27**, 809–817 (1990).
25) Potter, H., Weir, L. and Leder, P. Enhancer-dependent expression of human κ immunoglobulin genes introduced into mouse pre-B lymphocytes by electroporation. *Proc. Natl. Acad. Sci. USA*, **81**, 7161–7165 (1984).
26) Russo, C., Callegaro, L., Lanza, E. and Ferrone, S. Purification of IgG monoclonal antibody by caprylic acid precipitation. *J. Immunol. Methods*, **65**, 269–271 (1983).
27) Ey, P. L., Prowes, S. J. and Jenkin, C. R. Isolation of pure IgG1, IgG2a and IgG2b immunoglobulins from mouse serum using protein A-Sepharose. *Immunochemistry*, **15**, 429–436 (1978).
28) Greenwood, F. C., Hunter, W. M. and Glover, J. S. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. *Biochem. J.*, **89**, 114–117 (1963).
29) Scatchard, G. The attraction of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, **51**, 660–664 (1949).
30) Novotny, J., Handschumacher, M. and Haber, E. Location of antigenic epitopes on antibody molecules. *J. Mol. Biol.*, **189**, 715–721 (1986).