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Phage Display Cloning and Characterization of Monoclonal Antibody Genes and Recombinant Fab Fragment against the CD98 Oncoprotein

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The *Fab* gene of anti-CD98 heavy chain (h.c.) monoclonal antibody (mAb) HBJ127 was cloned and expressed as a recombinant Fab (rFab) fragment by means of a phage display system. The variable heavy and light chain genes of *HBJ127* were found to be derived from VOx-1 and IgVk8-30 germline, respectively. Extensive somatic mutation was found in the heavy chain complementarity determining region 2. rFab fragment was purified homogeneously from crude bacterial lysates by Ni-chelate chromatography in a yield of 71.4 μg from 100 ml of culture. rFab fragment was reactive with the cell surface of CD98-positive cells irrespective of tissues of origin, but not with CD98-negative cells. The recognition site of the rFab fragment was identical to that of mAb since the binding of rFab fragment to HeLaS$_3$ cells was completely inhibited by pretreatment with an excess of mAb. The relative affinity values of rFab fragment and mAb were found to be 0.11×10^8 and 0.35×10^8 M^{-1}, respectively. Three-fold lower affinity of rFab fragment may be due to the difference of valency of the antibody preparation. Cell growth inhibition *in vitro* by rFab fragment preincubated with anti-Fab suggests that the rFab fragment produced by cloned gene-bearing *Escherichia coli* was identical to the Fab part of HBJ127 mAb. These results show that a small fragment with antigen binding activity similar to that of the parent mAb can easily be prepared by using a phage display system. To our knowledge, this is a first report of the production of anti-CD98 h.c. rFab fragment.

Key words: Phage display — Monoclonal antibody — Recombinant Fab — CD98

CD98/GP125 is a heterodimeric protein with a relative molecular mass of 125-kDa consisting of a glycosylated 85-kDa heavy chain (h.c.) and a non-glycosylated 40-kDa light chain (l.c.), which are disulfide-linked.[1)] CD98 was identified originally as a cell surface antigen associated with lymphocyte activation defined by the 4F2 monoclonal antibody (mAb),[1)] and is expressed in proliferating normal tissues[2–5)] and in almost all tumor cells.[2, 5–7)] These findings suggest that CD98 is involved functionally in lymphocyte activation, cell proliferation and malignant transformation. In fact, a mAb against CD98 inhibited tumor cell growth[7)] and lymphocyte proliferation.[8)] Furthermore, we have recently demonstrated that CD98 h.c. cDNA-transfected murine fibroblasts showed various malignant phenotypes.[9–11)]

The clinical application of mAbs for diagnosis and therapy in human cancer has been a very active area of research. mAbs both alone and conjugated to isotopes, toxins, or anti-cancer drugs have been employed for clinical trials. The cell surface antigens expressed predominantly on tumor cells are suitable targets for antibody tumor therapy. Unlike typical receptor-type oncoproteins, such as members of the erbB/epidermal growth factor receptor family with a restricted tumor distribution,[12)] CD98 is overexpressed on the cell surface of almost all tumor cells irrespective of tissue of origin. Therefore, anti-CD98 h.c. mAbs would be promising for tumor immunotherapy. However, a drawback with the use of mAbs in the treatment of human diseases is their immunogenicity and the consequent elicitation of human anti-mouse antibodies following their repeated injection into patients.

Small fragments with the antigen binding activity, such as F(ab′)$_2$ and Fab would be preferable for clinical use because of their reduced immunogenicity and better pharmacokinetic properties[13)] compared with whole antibody. To prepare the small fragments of anti-CD98 h.c. mAb applicable for tumor imaging and targeting, we employed the phage display system for cloning of mAb genes and expression of recombinant Fab (rFab) fragment. Antibody

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phage display[14, 15] is a recently developed recombinant DNA technology for preparing recombinant antibody fragments from various immune sources, e.g. bone marrow cells, lymph node cells, peripheral blood lymphocytes, spleen cells, and hybridoma cells. It has been reported that recombinant human mAb fragments against viral pathogens[16–19] and autoantigens,[20–23] and recombinant murine mAb fragments against various tumor-associated antigens[24, 25] were successfully isolated by means of the phage display system. In this study, among the anti-CD98 h.c. mAbs, we selected HBJ127[5, 7, 8] as a starting material because it showed not only antigen binding activity, but also characteristic bioactivity including an inhibitory effect on cell growth[7, 9, 10] and lymphocyte proliferation.[8] The phage display cloning of *HBJ127* genes, molecular structural characterization of the cloned genes, and determination of the antigen binding activity and specificity of the rFab fragment expressed in the *Escherichia coli* (*E. coli*) are described here. To our knowledge, this is the first report regarding the production of anti-human CD98 h.c. rFab fragment.

MATERIALS AND METHODS

Cell culture HBJ127 (IgG_1, κ)-producing hybridoma cells were cultured in RPMI1640 medium supplemented with 100 U/ml of penicillin G, 100 μg/ml of streptomycin, 2 m*M* glutamine, and 10% heat-inactivated fetal bovine serum (FBS). Human carcinoma (ca.) cell lines, HeLaS_3 (cervix ca.), T24 (bladder ca.), MDA-MB-453 (breast ca.), MCF-7 (gastric ca.), LS-LM4 (colon ca.), Int407 human fetal kidney, ACHN (renal ca.), KNS human glioma and SV-T2 (SV40-transformed BALB/3T3 mouse fibroblastic cells) were cultured in minimum essential medium (MEM) with the additives mentioned above. Peer human leukemic cells were cultured in the medium used for hybridoma culture. All cell lines were maintained at 37°C in a humidified 5% CO_2 atmosphere.

Phage display cloning of immunoglobulin genes The construction of a Fab phage display combinatorial library from hybridoma cells was performed as previously described.[24] Briefly, total RNA of hybridoma cells (1×10^7) was extracted with an RNeasy Mini Kit (Qiagen, Tokyo). First-strand cDNA was then synthesized with a primer of oligo(dT)$_{15}$ using an AMV reverse transcriptase first-strand cDNA synthesis kit (Life Sciences, St. Petersburg, FL). PCR amplifications of the heavy chain (HC) Fd region and whole κ light chain (LC) were performed with the GeneAmp 2400 PCR system (Perkin-Elmer, Norwalk, CN) using the family-specific variable region and isotype-specific constant region primers designed based on the Kabat database[26] (Table I). Gel-purified PCR products were double-digested with restriction enzymes (*Spe*I/*Xho*I for HC and *Sac*I/*Xba*I for LC, respectively. All enzymes were purchased from Roche Diagnostics, Tokyo), and then ligated sequentially into the phage display vector pComb3

Table I. PCR Primers Used for Amplification of Mouse *IgG_1*, κ Genes

Primer	Sequence
(a) Primers used for amplification of heavy chain Fd region	
forward primer	
IA+IB	5′-SAG GTG CAG CTK CTC GAG TCA GGA CCT RGC-3′
IIA	5′-SAG GTY CAG CTG CTC GAG TCT GGA SCT GAG-3′
IIB	5′-CAG GTC CAR CTG CTC GAG YCT GGG GCT GAG-3′
IIC	5′-GAG GTT CAG CTG CTC GAG TCT GKG GCW GAG-3′
3A	5′-GAR GTG AAG GTG CTC GAG TCT GGR GGA GGC-3′
3B+3C	5′-GAR GTG AAG CTT CTC GAG TCT GGA GGW GGC-3′
3D	5′-GAR GTG CAG CTG CTC GAG GGK GGG GGA GGA-3′
reverse primer	
IgG1	5′-CGC GCG ACT AGT ACC ACA ATC CCT GGG CAC AAT TTT-3′
(b) Primers used for amplification of whole light chain	
forward primer	
kI	5′-GCG CGC GAG CTC GAC RTT GTG ATG WCA CAG TCT CCA TCC TYC-3′
kII	5′-GCG CGC GAG CTC GAT RTT KTG ATG ACC CAR ACT CCA CTC TCC-3′
kIII	5′-GCG CGC GAG CTC GAC ATT GTG CTG ACM CAR TCT CCW GCT TCC-3′
kIV	5′-GCG CGC GAG CTC SAA AWT GTK CTC ACC CAG TCT CCA GCA ATC-3′
kV	5′-GCG CGC GAG CTC GAY ATY CAG ATG ACM CAG WCT MCA TCC TCC-3′
kVI	5′-GCG CGC GAG CTC CAA ATT GTK CTC WCC CAG TCT CCA GCA ATC-3′
reverse primer	
κ	5′-GCG CGC TAT CTA GAA TTA ACA CTC ATT CCT GTT GAA GCT CTT-3′

Underlined is the introduced restriction site for cloning of the PCR product into pComb3.

(provided by Dr. Dennis R. Burton, The Scripps Research Institute, La Jolla, CA).[27] The constructed combinatorial library was electroporated into XL1-Blue cells (Stratagene, La Jolla, CA) with an *E.coli* pulser (Bio-Rad, Richmond, CA), then streaked onto LB agar plates containing 100 μg/ml of carbenicillin and 100 m*M* glucose. Single colonies were cultured in Super broth (SB) with 50 μg/ml of carbenicillin, and Fab-displayed phage (Fab-phage) clones were rescued by infection with VCSM13 helper phage (Stratagene). The Fab-phage clones from overnight culture were then examined for their binding activity to the corresponding antigen by indirect immunofluorescence assay as described below. The identity of the positive clones was determined by *Bst*NI (New England BioLab, Beverly, MA) fingerprinting.[21] The sequences of the cloned genes were confirmed by DNA sequencing using a 373A automated DNA sequencer (Perkin-Elmer). The primers SeqT_3 (5′-ATT AAC CCT CAC TAA AG-3′) and KEF (5′-GAA TTC TAA ACT AGC TAG TTC G-3′), both hybridizing to the (−)-strand, were used for sequencing variable heavy (V_H) and variable light (V_L) domains, respectively.

Expression and purification of rFab fragment The plasmid DNA of a positive clone was reengineered for rFab expression,[16, 27] and electroporated into XL-1 Blue cells. Single colonies were cultured in SB with 50 μg/ml of carbenicillin and 20 m*M* $MgCl_2$ at 37°C until an A_{600} of 1.0 was reached. Isopropyl β-D-thiogalactopyranoside (IPTG) (Roche Diagnostics) was then added at a final concentration of 1 m*M* and the culture was continued overnight at 30°C. The bacterial cells pelleted by centrifugation were resuspended in phosphate-buffered saline (PBS) containing 0.2 m*M* phenylmethylsulfonyl fluoride (PMSF) (Roche Diagnostics) and lysed by freeze-thawing four times. Debris was pelleted by centrifugation and the rFab fragment was finally obtained as a cleared supernatant. Purification of rFab fragment from supernatant was carried out under non-denaturing conditions using a Ni-NTA spin kit (Qiagen) according to the manufacturer's instructions. The purity of the rFab preparation was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 12.5% gel under non-reducing condition. The rFab band was then visualized by a Coomassie Brilliant Blue staining of the gel. The rFab concentration was determined by measuring absorbance at 280 nm.

Immunological assays The reactivity and specificity of rFab fragment against the CD98-positive and -negative cells were determined by an indirect immunofluorescence assay. Subconfluent cultures of $HeLaS_3$ ($CD98^+$), MDA-MB-453 ($CD98^+$), and SV-T2 ($CD98^-$) cells on Lab-Tek chamber slides (Nalgenunc International, Tokyo) were fixed with 4% (v/v) formalin-PBS. For unfixed cell staining, $HeLaS_3$ and MDA-MB-453 cells were detached from the culture flask with trypsin-EDTA (GIBCO-BRL, Lifetech Oriental, Tokyo), then washed once with PBS, and suspended in 100 μl of PBS (1×10^5 in each assay). Anti-c-erbB-2 rFab fragment was used as a negative control for testing the specificity of HBJ127 rFab fragment. The fixed or unfixed cells were successively treated with 5% (v/v) normal rabbit sera (Jackson ImmunoResearch, West Grove, PA) in PBS (NRS-PBS), rFab fragment (10 μg/ml in NRS-PBS), and FITC-labeled rabbit anti-mouse IgG $F(ab')_2$ (Jackson ImmunoResearch) (1:300 diluted in NRS-PBS). The samples were examined with a confocal laser microscope (LSM410, Carl Zeiss Japan, Tokyo).

For testing the identity of the recognition site of rFab fragment and mAb, freshly prepared Fab-phage (10^{11} pfu) was reacted with the formalin-fixed $HeLaS_3$ cells pretreated with 10 μg/ml of HBJ127 mAb. The bound Fab-phage was detected with rabbit anti-M13 (Stratagene) (1:500 in NRS-PBS) followed by FITC-labeled goat anti-rabbit IgG (Jackson ImmunoResearch) (1:300 in NRS-PBS). The samples were examined with LSM410 confocal laser microscope.

The relative affinity of rFab and mAb was determined by flow cytometry as previously described.[28] $HeLaS_3$ cells (2×10^5) were treated with rFab fragment or mAb that was serially diluted from 100 to 0.01 μg/ml in bovine serum albumin (BSA)-PBS and incubated on ice for 1 h. After washing three times with ice-cold PBS, the cells were treated with FITC-labeled rabbit anti-mouse IgG $F(ab')_2$ (1:100 in BSA-PBS). The cells were then incubated on ice for 1 h and washed three times with ice-cold PBS. The mean fluorescence intensity (MFI) of each sample was determined with a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA). A half of the minimum molar concentration that showed the peak MFI was considered as the dissociation constant (K_d). The affinity constant (K_a) was then calculated as the reciprocal of the K_d value.

The reactivity of rFab fragment against a panel of cultured human cell lines (see the cell culture section) was determined by flow cytometry. The cells in suspension were successively treated on ice with each rFab fragment (10 μg/ml in BSA-PBS), and FITC-labeled rabbit anti-mouse IgG $F(ab')_2$ (1:300 diluted in BSA-PBS). The MFI of individual cells was determined with a FACScan flow cytometer.

Cell growth inhibition assay The cell growth-inhibitory activity of rFab fragment was tested by using $HeLaS_3$ and MDA-MB-453 cells. The cells were seeded into flat-bottomed 96-well culture plates at 1×10^4 cells per well in triplicate, and cultured for 24 h at 37°C. rFab fragment alone or preincubated with a half-equimolar amount of rabbit anti-mouse IgG $F(ab')_2$ at a final rFab concentration of 50 μg/ml was then added and the cells were incubated for 72 h at 37°C. The same concentration of mAb was used as a positive control. The cells were incubated with Alamar Blue (Cosmo Bio, Tokyo) for the last 3 h of cul-

A

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
VOx-1	QVQLKESGPGLVAPSQSLSITCTVSGFSLT	SYGVH	WVRQPPGKGLEWLG	VIWAGGSTNYNSALMS	RLSISKDNSKSQVFLKMNSLQTDDTAMYYCAR		
HBJ127VH	..K.L.......Q.................	T..I.	....	...SN.RID..A.FI.	....T.........F......D....I.....	NVYDSLTWFTY	WGQGTLVTVSA

B

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
IgVk8-30	DIVMSQSPSSLAVSVGEKVTMSC	KSSQSLLYSSNQKNYLA	WYQQKPGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLTISSVKAEDLAVYYC	QQYYSYP	
HBJ127VL	..Q.T................T.	....	..............F	....	...............P..............F.	...T...T	FGGGTKLEIKRA

Fig. 1. Deduced amino acid sequences of V_H and V_L domains of HBJ127 with the closest known germline. (A) V_H sequences, (B) V_L sequences.

ture to measure the cell growth activity. The absorbances of the solution at 570 and 595 nm were measured with a Model 550 Microplate Reader (Bio-Rad) in the dual-wavelength measurement mode.

RESULTS

Phage display cloning of *HBJ127* genes The antibody *HC Fd* and *LC* genes were amplified by PCR with the primer sets shown in Table I using cDNA synthesized from total RNA of HBJ127-producing hybridoma cells. Amplification of the *HBJ127 HC* gene segment was achieved with the single primer set of IA+IB-IgG1. On the other hand, the *LC* gene was amplified with the primer sets kI-, kIII-, and kV-κ. The multiple amplification was due to the sequence similarity of the kI, kIII, and kV primers, and the kV primer was actually used for amplification, based on sequencing of the cloned *LC* gene segment. The amplified products were then pooled, gel-purified, and digested with restriction enzymes corresponding to the cloning sites. The combinatorial antibody library constructed by successive insertion of each LC and HC fragment into the phage display vector pComb3 contained 1×10^6 transformants. A total of 50 single colonies from the library were separately cultured, then the helper phage-rescued Fab-phage clones were tested for binding to the corresponding antigen by indirect immunofluorescence assay using formaldehyde-fixed $HeLaS_3$ cells. All positive clones in the library were found to be identical by *Bst*NI fingerprinting analysis (data not shown).

To elucidate the molecular structural characteristics of antigen-binding sites in HBJ127, the V_H and V_L domains of the selected clone were sequenced and analyzed. The deduced amino acid sequences together with the derived germ-line genes are shown in Fig. 1. HBJ127 V_H was found to be a member of the mouse VH2 family, while V_L belongs to the mouse Vk8 group I subgroup. The Ig BLAST analysis (http://www.ncbi.nlm.nih.gov/igblast/) revealed that HBJ127 V_H and V_L were derived from the germline genes of *VOx-1* (82.5% homology) and *IgVk8-30* (89.1% homology), respectively. The marked sequence difference (eight out of 16 residues) between the HC complementarity determining region (CDR) 2 and the corresponding region of the germline gene suggests that extensive somatic mutation had occurred in this region.

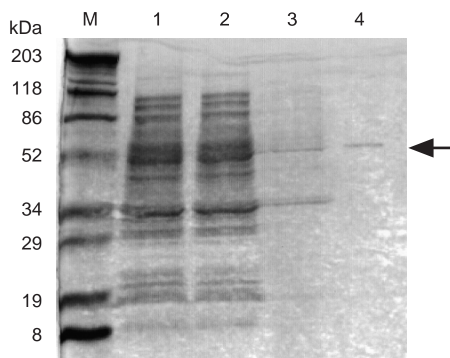

Fig. 2. Purification of HBJ127 rFab fragment from lysates by Ni-chelate chromatography. rFab fragment was purified from crude bacterial lysates by Ni-NTA spin kit, then the purity was checked by 12% SDS-PAGE under a non-reducing condition followed by Coomassie Brilliant Blue staining as described in "Materials and Methods." M, molecular weight marker; lane 1, bacterial lysates; 2, non-adsorbed fraction; 3, 20 m*M* imidazole wash fraction; 4, 250 m*M* imidazole eluate fraction. The arrow indicates the migrating position of rFab fragment.

Expression and purification of HBJ127 rFab fragment rFab fragment produced from the clones reengineered for rFab expression was recovered from the periplasm of IPTG-induced packed cells by freeze-thawing. The rFab fragment was then affinity-purified by Ni-chelate chromatography from the crude lysates, since we used the modified version of pComb3 (pComb3 #AP3/3H2) designed to express the hexa histidine tag next to the C terminal end of the HC insert. A single band of approximately 50-kDa with the definitive antigen binding activity was detected in the eluate fraction by SDS-PAGE after Coomassie Brilliant Blue staining (Fig. 2). The yield of the purified rFab fragment was 71.4 μg from 100 ml of culture.

Characterization of HBJ127 rFab fragment The antigen binding activity and specificity of the rFab fragment were evaluated by means of an indirect immunofluorescence assay. The rFab fragment reacted mainly with the cell membrane fraction rather than the intracellular fraction of the fixed $HeLaS_3$ and MDA-MB-453 cells (Fig. 3, A and B). The staining pattern of the corresponding unfixed cells showed that the rFab fragment could bind to the surface of living cells (Fig. 3, D and E). The binding of the rFab fragment to $HeLaS_3$ and MDA-MB-453 cells was CD98-specific reaction since the fragment showed no reactivity to CD98-negative SV-T2 mouse fibroblastic cells (Fig. 3C) and rFab fragment directed to the c-erbB-2 product (manuscript in preparation) showed no reactivity to the antigen-negative $HeLaS_3$ cells (Fig. 3F). The recognition sites of rFab and mAb were found to be identical since the binding of Fab-phage to $HeLaS_3$ cells was completely inhibited by the pretreatment of mAb (Fig. 4A). Fab-phage (Fig. 4B) and mAb (Fig. 4C) alone each showed the definitive reactivity to $HeLaS_3$ cells. The rela-

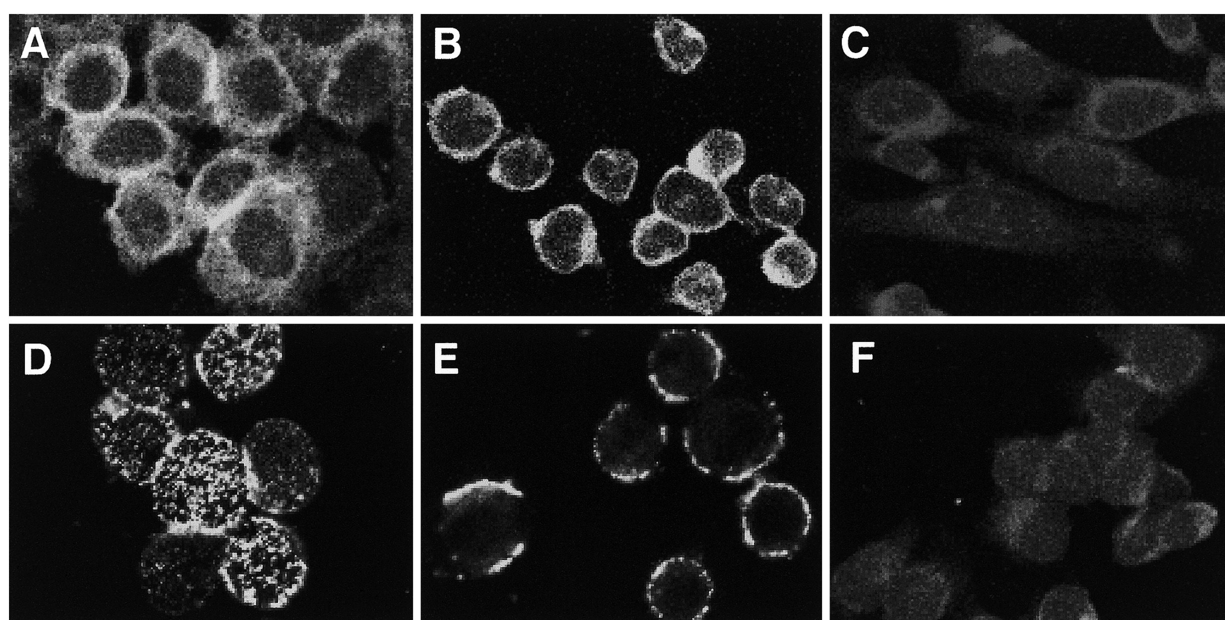

Fig. 3. Indirect immunofluorescence staining with HBJ127 rFab fragment in CD98-positive and -negative cultured cell lines. A and B, rFab fragment staining of fixed and unfixed $HeLaS_3$ cells ($CD98^+$); C, rFab fragment staining of fixed SV-T2 cells ($CD98^-$); D and E, rFab fragment staining of fixed and unfixed MDA-MB-453 cells ($CD98^+$); F, rFab fragment against c-erbB-2 product staining of fixed $HeLaS_3$ cells (c-erbB-2^-).

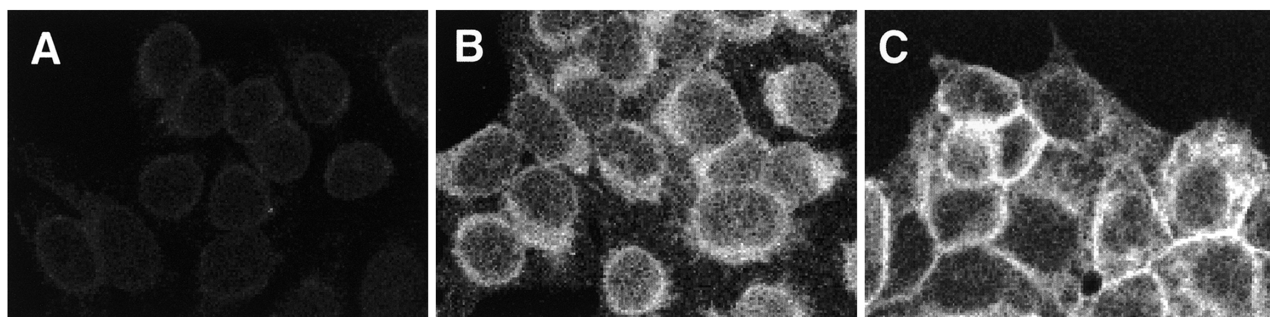

Fig. 4. Inhibition of HBJ127 rFab-displayed phage binding to $HeLaS_3$ cells by HBJ127 mAb. rFab-phage was reacted with $HeLaS_3$ cells pretreated with mAb, then bound rFab-phage was detected by rabbit anti-M13 followed by FITC-goat anti-rabbit IgG as described in "Materials and Methods." A, rFab-phage staining of mAb-pretreated $HeLaS_3$ cells; B, rFab-phage staining of $HeLaS_3$ cells; C, mAb staining of $HeLaS_3$ cells.

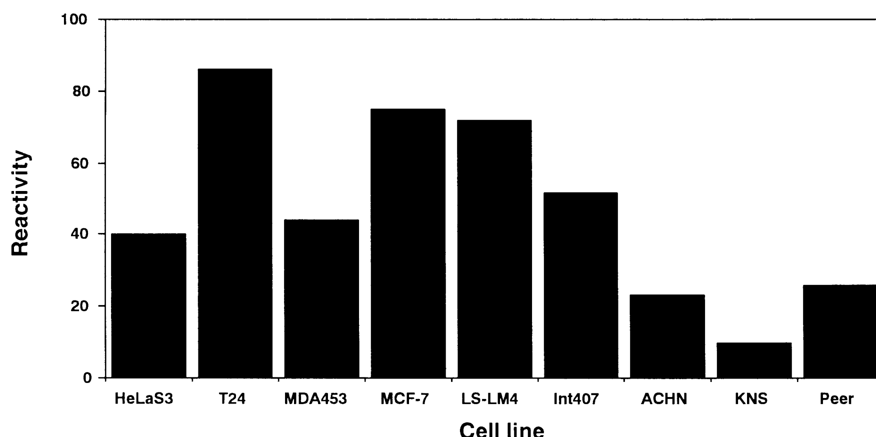

Fig. 5. Reactivity of HBJ127 rFab fragment against a panel of cultured human cell lines. Unfixed cells were reacted with rFab fragment, then analyzed for mean fluorescence intensity with a FACScan flow cytometer as described in "Materials and Methods."

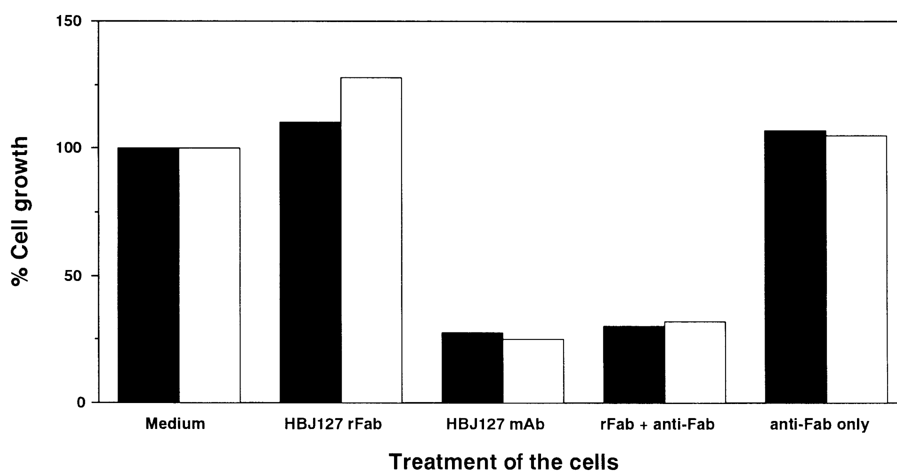

Fig. 6. Effect of HBJ127 rFab fragment on *in vitro* cell growth of $HeLaS_3$ and MDA-MB-453 cells. Cells (1×10^4) were cultured with rFab fragment (HBJ127 rFab), mAb (HBJ127 mAb), rFab fragment pretreated with rabbit anti-mouse IgG $F(ab')_2$ (rFab+anti-Fab), and rabbit anti-mouse IgG $F(ab')_2$ (anti-Fab only), respectively, for 72 h at 37°C. The cell growth activity was then evaluated by Alamar Blue assay as described in "Materials and Methods." ■ $HeLaS_3$, □ MDA-MB-453.

tive affinity of rFab fragment and mAb was then determined by flow cytometry. The calculated affinity constants of rFab fragment and mAb were 0.11×10^8 and 0.35×10^8 M^{-1}, respectively. Although the apparent affinity of the rFab fragment was approximately three-fold lower than that of mAb, their affinity may be considered in the same range if the valency of each antibody preparation is taken into consideration. The reactivity of rFab fragment against a panel of human culture cell lines was determined by flow cytometry (Fig. 5). rFab fragment showed definite reactivity against all tested cell lines irrespective of tissue of origin. This is consistent with the notion that all cycling cell lines express CD98 h.c. on their cell surface.[2–7)]

Cell growth-inhibitory activity of HBJ127 rFab fragment HBJ127 mAb shows growth-inhibitory activity against antigen-expressing cells *in vitro*.[8)] rFab fragment was then examined for activity to inhibit cell growth using $HeLaS_3$ and MDA-MB-453 cells, both of which express a moderate amount of CD98 h.c. protein on their cell surface. Although rFab fragment alone showed no growth-inhibitory activity against the tested cell lines, rFab fragment preincubated with rabbit anti-mouse IgG $F(ab')_2$ showed cell growth-inhibitory activity to the same extent as whole mAb (Fig. 6). This implies that the rFab fragment produced by cloned gene-bearing *E. coli* was actually identical to the Fab part of HBJ127 mAb, and the

binding of HBJ127 mAb to the epitope followed by cross-linking of the antigen is indispensable for eliciting the cell growth-inhibitory activity.

DISCUSSION

In this report, we describe the cloning of a post-rearranged antibody cDNA and expression of *Fab* genes to afford a recombinant protein from anti-CD98 h.c. mAb, HBJ127-producing hybridoma cells by using a phage display system. Recently, phage display cloning has become a general strategy for preparing recombinant antibodies originating from humans,[16–23] mice,[24, 25] and other species.[29, 30] Antibody genes are expressed as the Fab or single chain Fv (scFv) form using suitable vectors and cloning systems. We used the pComb3 system reported by Barbas *et al.*[27] This system was optimized for expression of antibody genes in the Fab form and for the targeting of recombinant proteins to the periplasmic space of host cells by the pelB leader sequence. The HC Fd and LC fragments are thought to associate by the formation of a disulfide bond in the periplasmic space under reducing conditions. The expressed rFab fragment is therefore more natural than the scFv produced by combining the V_H and V_L domains using a polypeptide linker, and possesses the original mAb specificity. The recombinant proteins are generally produced as cytoplasmic inclusion bodies in the host cells. Refolding of recombinant proteins by dissociating reagents, such as urea, would be necessary, but a decrease in the antigen-binding activity can be anticipated. Specifically designed systems should be employed for the detection of the antigen-bound scFv fragments. On the other hand, rFab fragments produced with the pComb3 system can easily be recovered by sonication or freeze-thawing of host cells with a minimum loss of antigen binding activity because the recombinant proteins are accumulated in the periplasmic space of the host cells. Commercially available secondary antibody, e.g. anti-mouse IgG $F(ab')_2$, can be used for the detection of antigen-bound rFab fragments. For these reasons, the pComb3 system used in this study may be better suited for the cloning and expression of *mAb* genes than the scFv production systems.

The V_H domain of an antibody is known to play a major role in antigen recognition and binding.[19, 31] Somatic mutations in V segments during B cell maturation are indispensable for rearranged antibodies to obtain high specificity and affinity.[32, 33] HCDR3 located at the junction of V-D-J segments is also thought to be important for antigen recognition because of its diversity in length and amino acid usage.[34] HBJ127 V_H was derived from VOx-1 with 87.3% and 82.5% homology in the nucleotide and amino acid sequence, respectively. The relatively low homology between them showed that extensive somatic mutations had occurred in the V segment, especially in HCDR2 (8 out of 16 residues), during the affinity maturation of the antibodies. No biased amino acid length or usage was seen in HCDR3 of HBJ127 mAb. These observations indicate that HCDR2 is the major determinant for antigen-recognition by HBJ127 mAb. HBJ127 mAb with increased specificity and affinity may be obtained by introduction of point mutations in HCDR2.

We demonstrated that rFab fragment produced by a phage display system showed affinity and specificity almost equal to those of the parent mAb. Minimal loss of antigen binding activity during expression in *E.coli*, recovery from cells, and purification by Ni-chelate chromatography would be expected. We also demonstrated that rFab fragment preincubated with anti-$F(ab')_2$ showed the cell growth-inhibitory activity. This preliminary experiment suggests that the use of HBJ127 rFab genetically fused with the human IgG Fc casette[35] for cancer immunotherapy might be feasible. For clinical application of mAb, human antibodies against tumor antigens would be the best, though it is very difficult to isolate them because of the low immunogenicity of tumor antigens in humans. Figini *et al.* reported that human antibody against the folate-binding protein (FBP), a cell surface antigen that is overexpressed in many human ovarian carcinomas, has been isolated using the genes of high-affinity mouse mAb against FBP as guides for selection, termed guided selection.[36] Our final goal is to isolate the recombinant human antibody fragment against CD98 h.c. The *HBJ127 mAb* genes cloned in this study would be applicable for guided selection. Construction of phage display combinatorial libraries from a CD98-positive MOLT lymphoma patient's bone marrow cells and its application to the guided selection with *HBJ127 mAb* genes for isolation of recombinant human antibodies against CD98 h.c. is in progress.

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