Jpn. J. Cancer Res. 83, 515–521, May 1992

Mouse-Human Chimeric Antibody MH171 against the Multidrug Transporter P-Glycoprotein

Keiko Ariyoshi,[1,2] Hirofumi Hamada,[1] Mikihiko Naito,[3] Yuji Heike,[1] Hiroyuki Seimiya,[3] Kayoko Maezawa[1] and Takashi Tsuruo[1,3]

[1]*Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 1-37-1 Kami-Ikebukuro, Toshima-ku, Tokyo 170,* [2]*Hoechst Japan Ltd. Pharma Research Laboratories, 1-3-2 Minami-dai, Kawagoe-shi, Saitama 350 and* [3]*Institute of Applied Microbiology, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113*

We have developed a mouse-human chimeric antibody MH171, in which the antigen-recognizing variable regions of the mouse monoclonal antibody MRK17 are joined with the constant regions of human IgG1 antibodies. The MRK17 recognizes specifically the multidrug transporter P-glycoprotein and inhibits the growth of human multidrug resistant (MDR) tumor cells *in vitro* and in the xenograft nude mouse model system. The established chimeric MH171 antibody forms an apparently intact IgG composed of heavy and light chains covalently assembled via disulfide bonds in sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis and is specific to MDR cell lines with a similar affinity to the original mouse MRK17. MH171 also displays strong antibody-dependent cell-mediated cytotoxicity to the target cells *in vitro*, when human mononuclear cells are used as effector cells. The chimeric antibody against P-glycoprotein, MH171, should be a useful agent in the treatment of human drug-resistant tumors.

Key words: Mouse-human chimeric antibody — P-glycoprotein — Multidrug-resistance — MRK17 — MH171

Cross-resistance of tumors to a variety of chemotherapeutic drugs such as doxorubicin (adriamycin), *Vinca* alkaloids, and actinomycin D is a major problem in cancer chemotherapy.[1,2] The gene responsible for multidrug resistance, termed *MDR*1, encodes a membrane glycoprotein (P-glycoprotein) that acts as a pump to transport various cytotoxic drugs out of the cell.[3] The P-glycoprotein has been shown to bind anticancer drugs[4,5] and to be an ATPase[6,7] localized at the plasma membrane of multidrug-resistant cells.[8,9] Transfection of cloned *MDR*1 sequences confers multidrug resistance on sensitive cells.[10–12]

The level of *MDR*1 gene expression was found to be elevated in intrinsically drug-resistant cancers of the colon, kidney, and adrenal as well as in some tumors that acquired drug resistance after chemotherapy.[13–15] Although the enhanced expression of P-glycoprotein is not the only mechanism of multidrug resistance, the selective killing of tumor cells expressing P-glycoprotein could be important for cancer therapy, because elevated P-glycoprotein levels appear to be involved in both acquired multidrug resistance and intrinsic drug resistance in human cancer.

In an effort to devise an effective treatment for human drug-resistant cancers, we developed monoclonal antibodies, MRK16 and MRK17, reactive to the multidrug transporter P-glycoprotein.[16] These monoclonal antibodies prevented tumor development in athymic mice inoculated s.c. with drug-resistant human ovarian cancer cells.[17] However, the mouse monoclonal antibodies, as foreign proteins, may evoke counteracting immune reactions that could reduce their effectiveness and may also cause allergic reactions in the patients.[18,19] We previously developed a mouse-human chimeric antibody MH162, in which the antigen-recognizing variable regions of MRK16 were joined with the constant regions of human IgG1.[20] The chimeric MH162 antibody gave higher ADCC[4] activity against multidrug-resistant tumor cells than the mouse MRK16 antibody when human effector cells were used. The MRK16 partially inhibited drug efflux of human multidrug-resistant tumor cells *in vitro* and did not inhibit the growth. However, the other antibody MRK17 did inhibit the growth of the same cell line.[17] Therefore, MRK17 and its chimeric antibody may exert additional therapeutic effects by another mechanism on the treatment of human multidrug-resistant tumors. In this study, we constructed recombinant chimeric antibodies in which the antigen-recognizing vari-

[1] To whom correspondence and requests for reprints should be addressed.

[4] The abbreviations used are: ADCC, antibody-dependent cell-mediated cytotoxicity; V, variable; C, constant; H, heavy; Ecogpt, *Escherichia coli* xanthine-guanine phosphoribosyl transferase gene; neo, Tn5 neomycin resistance gene; Sp2/0, Sp2/0-Ag14; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; MDR, multidrug-resistance; ATCC, American Type Culture Collection.

able regions of MRK17 are joined with the constant regions of human antibodies.[21, 22]

MATERIALS AND METHODS

Vectors, clones, probes, and cells λZAP[23] and λEMBL-3[24] were used as *Eco*RI and *Bam*HI vectors, respectively. pSV2-HG1gpt and pSV2-HCκneo were constructed as described previously.[25] Mouse Jκ and J_H probes were provided by Drs. K. Miura and Y. Kurosawa.[25] The hybridoma that produced the monoclonal antibody MRK17 was generated and maintained as described previously.[16] Human drug-resistant cell lines (K562/ADM and 2780^{AD}), their parent drug-sensitive cell lines (K562 and A2780, respectively), and mouse myeloma cell Sp2/0 were maintained as described previously.[26] Anti-phosphorylcholine mouse-human chimeric antibody was a gift from Drs. Miura and Kurosawa.[25]

Cloning of kappa and heavy chain gene cDNAs MRK17 kappa and heavy chain cDNA clones were isolated essentially as described previously.[27] Briefly, the cDNA library of the MRK17-producing hybridoma cells was synthesized as described,[28] ligated with the *Eco*RI site of the λZAP vector, and packaged into λ phage. The cDNA library was screened with the mouse kappa and heavy constant region genomic DNA probes.[20] Plaque hybridization was performed according to the Benton-Davis method.[29] The selected cDNA clones were subcloned into the *Eco*RI site of the pBluescript SK M13— vector using *in vivo* excision technique as recommended by the supplier (Stratagene). Partial nucleotide sequences of the variable regions of the kappa and heavy chain gene of MRK17 were determined by the dideoxy-termination method using T7 DNA polymerase (Sequenase™, United States Biochemical Corp.).

Cloning of genomic DNA The genomic DNA library of the MRK17-producing hybridoma was constructed by the *Mbo*I partial digestion method.[30] DNAs were eluted from the relevant regions separated on agarose gel, ligated with the *Bam*HI site of the λEMBL3, and packaged into λ phage. The rearranged kappa and heavy chain V(D)J-region genes were respectively screened by the double selections using specific J-region probes[25] and variable region probes obtained from the cDNA clones described above.

DNA transfection of mouse Sp2/0 myeloma cells Two hundred μg each of plasmids pSV2-VH17-HG1gpt and pSV2-Vκ17-HCκneo (see Fig. 2) were cotransfected into 10^7 mouse Sp2/0 cells (CRL1581, ATCC) by electroporation.[31, 32] Transformants were selected in RPMI1640 medium supplemented with 10% fetal bovine serum and 0.5 mg/ml G418 (GIBCO, Grand Island, NY). Human antibody in the growth medium was detected by enzyme-linked immunosorbent assay.[16, 26]

Isolation of chimeric antibody Antibody-producing cells were grown in RPMI 1640 medium supplemented with 1.0% fetal bovine serum, which had been precleared of Protein A-binding bovine immunoglobulin by affinity chromatography using Protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden).[33] Purification of the antibody was carried out as described previously,[34] using Protein A-Sepharose CL-4B affinity chromatography.

Cell-binding radioimmunoassay Mouse-human chimeric antibody was labeled with [^{14}C]leucine as described.[35] Briefly, tens of millions of the myeloma cells producing MH171 were labeled by metabolic incorporation of 25 μCi of L-[*U*-^{14}C]leucine (New England Nuclear, 344.0 mCi/mmol) per 30 ml of leucine-free RPMI 1640 medium containing 10% dialyzed fetal bovine serum (which had been precleared of Protein A-binding bovine immunoglobulin as described above) for 72 h at 37°C in a CO_2 incubator. Labeled MH171 was purified as described above. Cell-binding radioimmunoassay was performed essentially as described previously.[16] Competition inhibition cell-binding radioimmunoassay was carried out as described.[36] A million K562/ADM cells or other cell lines were preincubated for 30 min at 37°C with various concentrations of unlabeled blocking antibodies in 100 μl of phosphate-buffered saline (PBS, 0.02 *M* sodium phosphate-0.15 *M* NaCl, pH 7.4) supplemented with 5% fetal bovine serum. Then 3×10^3 dpm of [^{14}C]leucine-labeled MH171 antibody in 100 μl of PBS supplemented with 5% fetal bovine serum was added. After incubation at 37°C for 60 min, the cells were washed with ice-cold PBS 3 times and the radioactivity was counted in a liquid scintillation counter.

SDS-PAGE Denaturing gel electrophoresis was performed according to Laemmli[37] with a 4–20% polyacrylamide linear gradient gel, and gels were stained with 0.05% Coomassie brilliant blue.

ADCC Mononuclear cells from peripheral blood of normal volunteers were used as the effector cell source. Target cells were labeled with ^{51}Cr, as described.[17] A cell suspension (100 μl) containing 10^4 labeled target 2780^{AD} cells was incubated at 37°C for 30 min with 0.1 μg/ml of antibody in a 96-well microculture plate. Then, 100 μl of cell suspension containing effector mononuclear cells was added to each well at different E/T ratios. The plate was 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 scintillation counter. Determination was carried out in triplicate. The percentage of specific cytolysis was calculated from ^{51}Cr release of test samples and control samples, as follows:

$$\% \text{ of specific release} = [(E-S)/(M-S)]\times 100,$$

where E is experimental release (cpm in supernatant

from target cells incubated with effector cells and experimental antibody), S is spontaneous release (cpm in supernatant from target cells incubated with medium alone), and M is maximum release (cpm released from target cells lysed with 1% Triton X-100).

RESULTS

Construction of the chimeric heavy chain gene The rearranged variable region (V-D-J) gene of mouse heavy chain was cloned from the MRK17-producing hybridoma cells as a 15-kb *Sal*I fragment using the λEMBL3 library (Fig. 1A). The variable region gene had rearranged to the J4 segment. The mouse variable region gene was used as a 6.5-kb *Eco*RI fragment for constructing a chimeric heavy-chain gene (Fig. 1A). The mouse variable region gene was joined to a 5′ site of the human constant region in the same transcriptional direction, resulting in construction of pSV2-VH17-HG1gpt (Fig. 2A).

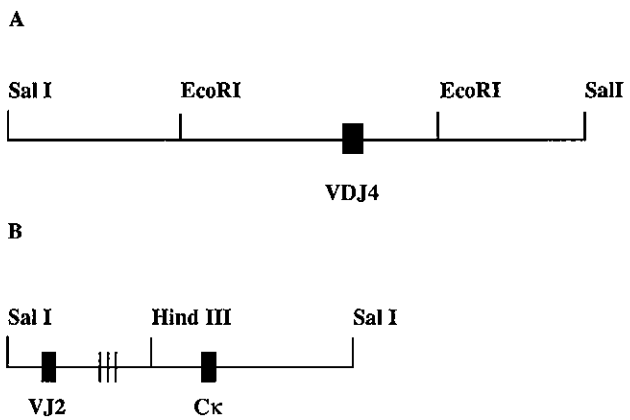

Fig. 1. Restriction map of rearranged genomic DNAs. Restriction enzyme maps of rearranged heavy (A) and kappa (B) genomic DNAs cloned in λEMBL3 are shown.

Construction of the chimeric light chain gene The light chain variable region gene was cloned from genomic DNA of MRK17-producing hybridoma as a 9-kb *Sal*I fragment using the λEMBL3 library. The variable region gene had rearranged to the J2 segment (Fig. 1B). The mouse variable region gene was trimmed to a 4-kb *Sal*I/*Hin*dIII fragment and was subcloned into the *Sal*I/*Hin*dIII site of pBluescript SK M13+. The resultant 4-kb fragment of mouse variable region was cut out by *Not*I/*Sal*I digestion and was cloned into the multicloning site of the pSV2-HCκneo.[20] Thus, the mouse light-chain variable region gene was joined to a 5′ site of the human constant region in the same transcriptional direction, resulting in construction of pSV2-Vκ17-HCκneo (Fig. 2B).

Transformation of mouse myeloma cells Sp2/0, a non-producer mouse myeloma cell line, was cotransfected with the chimeric heavy- and light-chain genes using electroporation methods.[31, 32] The transformed cells were selected with G418. The resultant stable transformants, which were obtained about 2 wk after the electroporation, were screened to select clones that produced the antibody specific to the multidrug-resistant cell line, K562/ADM. Screening was done by enzyme-linked immunosorbent assay, using parent K562 cells as negative control.[16] Five positive clones producing chimeric anti-

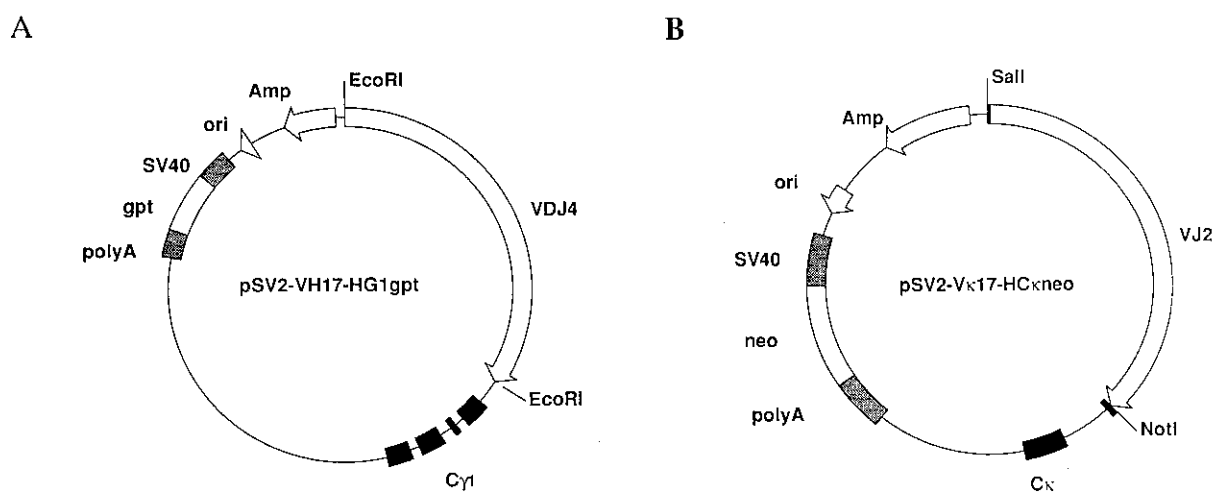

Fig. 2. Construction of plasmids: pSV2-VH17-HG1gpt (A) and pSV2-Vκ17-HCκneo (B). Abbreviations: ori, pBR322 ori; Amp, β-lactamase; SV40, SV40 promoter; polyA, polyA attachment signal.

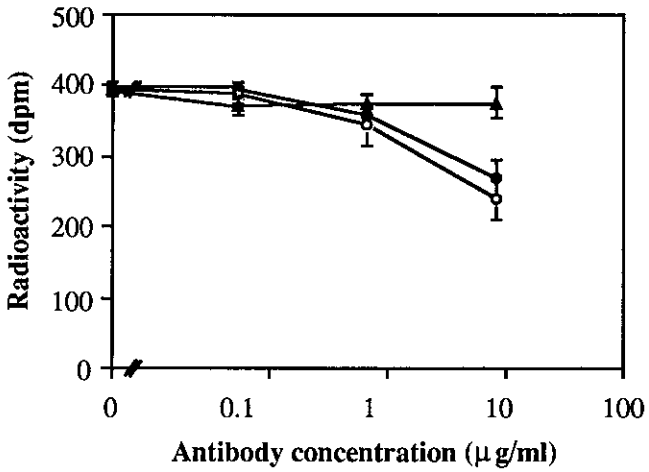

Fig. 3. Antibody-competitive binding assays. K562/ADM cells were incubated with various concentrations of unlabeled blocking antibodies before addition of [^{14}C]leucine-labeled MH171 chimeric antibody. Blocking antibodies used were MH171 (●), MRK17 (○), and the anti-phosphorylcholine mouse-human chimeric antibody (▲). Values represent the mean of triplicate determinations, and the bars represent SD.

Table I. Cell-binding Radioimmunoassay Showing Specific Binding of MH171 to K562/ADM and 2780^{AD}

	K562	K562/ADM	A2780	2780^{AD}
Cell-bound radioactivity (dpm)	39±3[a]	299±7	47±1	255±16

a) Mean±SD.

body were established from 2×10^7 cells. Products of one of the recloned transformants, designated MH171, were used for further analysis. The Sp2/0 transformants yielded 1 to 5 μg/ml of chimeric antibody in the medium supplemented with 1% serum, which had been deprived of Protein A-binding immunoglobulins. The apparent avidity of the chimeric antibody (MH171) to the K562/ADM cell antigen was similar to that of the mouse antibody (MRK17), as determined by enzyme-linked immunosorbent assay, and also by competition inhibition cell-binding radioimmunoassay (Fig. 3).

Tests on antibody specificity The antibody (MH171) specificity was tested by enzyme-linked immunosorbent assay (data not shown) and also by cell-binding radioimmunoassay. As shown in Table I, the chimeric antibody bound to multidrug-resistant cell lines K562/ADM and 2780^{AD}, but not to the parent drug-sensitive lines K562 or A2780. Thus, MH171 has the same binding specificities as MRK17, which was reported previously.[16, 38]

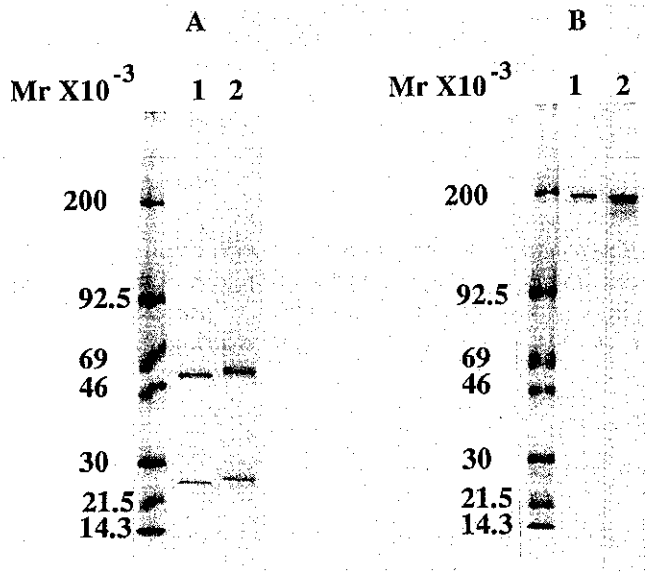

Fig. 4. SDS-PAGE analysis of mouse-human chimeric antibody MH171. Monoclonal antibody MRK17 (lane 1) and MH171 (lane 2), 0.5 μg each, were subjected to SDS-PAGE analysis either with (A) or without (B) 2-mercaptoethanol treatment. Molecular weight markers were obtained from Amersham, Japan.

SDS-PAGE analysis of the chimeric antibody The chimeric antibody MH171 was purified to apparent homogeneity by single-step affinity chromatography using Protein A-Sepharose (Fig. 4). Reducing SDS-PAGE analysis of chimeric antibody revealed two bands at 56 and 25 kDa, which corresponded to the heavy chain and light chain of the antibody, respectively (Fig. 4A). Under nonreducing conditions, a major band of MH171 was observed at 190 kDa (Fig. 4B), which is apparently slightly higher than that of MRK17.

ADCC by the chimeric antibody The chimeric MH171 exhibited significantly higher cytotoxicity than mouse MRK17 when human mononuclear cells were used as effector cells. This tendency was observed even at the low E/T ratio of 1.25:1 (Fig. 5). The chimeric MH171 or the mouse MRK17 showed no significant specific ADCC activity toward the drug-sensitive A2780 cells (data not shown).

DISCUSSION

Monoclonal antibodies directed to cell surface markers may be useful in tumor imaging and cancer therapy, not only to deliver a drug, toxin, or radioactive compound specifically to the target cells, but also to induce immune reactions that prevent tumor development by activation of complement and ADCC of lymphocytes and macro-

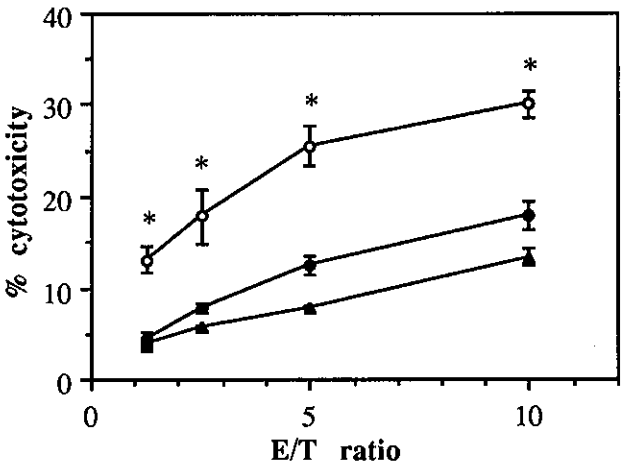

Fig. 5. Comparison of antibody-dependent cell-mediated cytotoxicity (ADCC) between murine and chimeric antibodies. ADCC activities of human mononuclear cells against human multidrug resistant ovarian cells, $2780^{A\,D}$, were measured with MH171 (○), MRK17 (●), and without antibody (▲), as described in "Materials and Methods." The concentration of each antibody was 0.1 μg/ml in these experiments. * : At each E/T ratio, means with MH171 are significantly different from means with MRK17 by t test ($P<0.005$).

phages *in vivo*. Mouse monoclonal antibodies MRK16 and MRK17 have the ability to recognize the cell surface domains of the multidrug transporter P-glycoprotein.[16] In previous[20] and current studies, we have successfully developed mouse-human chimeric antibodies of these murine-type monoclonal antibodies. We developed and characterized an IgG1-type chimeric antibody, since human IgG1 isotype is highly effective in both complement and cell-mediated killing.[39,40] The resultant chimeric IgG1 antibodies possessed similar affinity and binding specificity to the respective original murine types (Fig. 3), and showed higher ADCC activities than the murine types (Fig. 5). These results suggest that the chimerization of murine monoclonal antibodies may provide stronger effector functions *in vivo*. It remains to be studied whether the present chimeric antibodies, MH171 and MH162, are actually more effective in killing tumors and less immunogenic in appropriate models, as reported previously.[41,42]

Since our monoclonal antibodies are not cross-reactive to mouse P-glycoprotein,[16,17] it is impossible to predict undesirable side effects of these antibodies in the murine model systems.[17] In humans, the anti-P-glycoprotein antibody might produce undesirable side effects on normal organs and tissues including adrenal, kidney, liver, colon, and capillary endothelial cells in the brain where P-glycoproteins are expressed.[14,43-46] Recently, transgenic mice that express the human multidrug resistance gene (*MDR*1) in bone marrow have been developed.[47] These transgenic mice provide a rapid and reliable model to assess the activity of MDR-reversing agents *in vivo*.[48] Developmemt of animal models to assess side effects of these antibodies would be rewarding.

Human monoclonal antibodies have more clinical value than murine monoclonal antibodies, which often evoke counteracting immune responses that, in turn, generate human anti-mouse antibodies that may reduce their effectiveness and may also cause allergic side effects in the patients. Recently, several studies on reshaped humanized antibodies have been conducted to overcome these problems.[49-52] These super-chimerized antibodies consist of mostly human antibody amino acid sequences except for predicted binding sites (only six complementarity-determining regions) that are derived from the heavy and light chain variable regions of the rodent antibodies. Some of them are less immunogenic and have a longer half-life than the murine-type and chimeric-type antibodies in phase I clinical study. Although some technical difficulties remain to be solved, such as lowered affinity and productivity, humanization of murine-type antibodies including chimerization is one of the most important and promising approaches to improve the efficacy of monoclonal antibodies in cancer immunotherapy.

ACKNOWLEDGMENTS

This work was supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, the Ministry of Health and Welfare, and the Cell Science Research Foundation, Japan. We thank Drs. Y. Kurosawa and K. Miura for providing materials, Drs. K. Ono and E. Amann for helpful discussions, and Drs. E. Konz and H. B. Maruyama for their support and encouragement.

(Received December 14, 1991/Accepted February 25, 1992)

REFERENCES

1) Riordan, J. R. and Ling, V. Genetic and biochemical characterization of multidrug resistance. *Pharmacol. Ther.*, **28**, 51–75 (1985).
2) Tsuruo, T. Mechanisms of multidrug resistance and implications for therapy. *Jpn. J. Cancer Res.*, **79**, 285–296 (1988).
3) Roninson, I. B. "Molecular and Cellular Biology of Multidrug Resistance in Tumor Cells" (1991). Plenum Publishing

Corp., New York.
4) Safa, A. R., Glover, C. J., Meyers, M. B., Biedler, J. L. and Felsted, R. L. Vinblastine photoaffinity labeling of a high molecular weight surface membrane glycoprotein specific for multidrug-resistant cells. *J. Biol. Chem.*, **261**, 6137–6140 (1986).
5) Cornwell, M. M., Safa, A. R., Felsted, R. L., Gottesman, M. M. and Pastan, I. Membrane vesicles from multidrug-resistant human cancer cells contain a specific 150- to 170-kDa protein detected by photoaffinity labeling. *Proc. Natl. Acad. Sci. USA*, **83**, 3847–3850 (1986).
6) Hamada, H. and Tsuruo, T. Purification of the 170- to 180-kilodalton membrane glycoprotein associated with multidrug resistance: 170- to 180-kilodalton membrane glycoprotein is an ATPase. *J. Biol. Chem.*, **263**, 1454–1458 (1988).
7) Hamada, H. and Tsuruo, T. Characterization of the ATPase activity of the *Mr* 170,000 to 180,000 membrane glycoprotein (P-glycoprotein) associated with multidrug resistance in K562/ADM cells. *Cancer Res.*, **48**, 4926–4932 (1988).
8) Willingham, M. C., Richert, N. D., Cornwell, M. M., Tsuruo, T., Hamada, H., Gottesman, M. M. and Pastan, I. Immunocytochemical localization of P170 at the plasma membrane of multidrug-resistant human cells. *J. Histochem. Cytochem.*, **35**, 1451–1456 (1987).
9) Sugawara, I., Kataoka, I., Morishita, Y., Hamada, H., Tsuruo, T., Itoyama, S. and Mori, S. Tissue distribution of P-glycoprotein encoded by a multidrug-resistant gene as revealed by a monoclonal antibody, MRK16. *Cancer Res.*, **48**, 1926–1929 (1988).
10) Shen, D-W., Fojo, A., Roninson, I. B., Chin, J. E., Soffier, R., Pastan, I. and Gottesman, M. M. Multidrug resistance of DNA-mediated transformants is linked to transfer of the human *mdr*1 gene. *Mol. Cell. Biol.*, **6**, 4039–4045 (1986).
11) Gros, P., Neriah, Y. B., Croop, J. M. and Housman, D. E. Isolation and expression of a complementary DNA that confers multidrug resistance. *Nature*, **323**, 728–731 (1986).
12) Ueda, K., Cardarelli, C., Gottesman, M. M., and Pastan, I. Expression of a full-length cDNA for the human "*MDR*1" gene confers resistance to colchicine, doxorubicin, and vinblastine. *Proc. Natl. Acad. Sci. USA*, **84**, 3004–3008 (1987).
13) Bell, D. R., Gerlach, J. H., Kartner, N., Buich, R. N. and Ling, V. Detection of P-glycoprotein in ovarian cancer: a molecular marker associated with multidrug resistance. *J. Clin. Oncol.*, **3**, 311–315 (1985).
14) Fojo, A. T., Ueda, K., Slamon, D. J., Poplack, D. G., Gottesman, M. M. and Pastan, I. Expression of a multidrug-resistance gene in human tumors and tissues. *Proc. Natl. Acad. Sci. USA*, **84**, 265–269 (1987).
15) Tsuruo, T., Sugimoto, Y., Hamada, H., Roninson, I., Okumura, M., Adachi, K., Morishima, Y. and Ohno, R. Detection of multidrug resistance markers, P-glycoprotein and *mdr*1 mRNA, in human leukemia cells. *Jpn. J. Cancer Res.*, **78**, 1415–1419 (1987).
16) Hamada, H., and Tsuruo, T. Functional role for the 170- to 180-kDa glycoprotein specific to drug-resistant tumor cells as revealed by monoclonal antibodies. *Proc. Natl. Acad. Sci. USA*, **83**, 7785–7789 (1986).
17) Tsuruo, T., Hamada, H., Sato, S. and Heike, Y. Inhibition of multidrug-resistant human tumor growth in athymic mice by anti-P-glycoprotein monoclonal antibodies. *Jpn. J. Cancer Res.*, **80**, 627–631 (1989).
18) Meeker, T. C., Lowder, J. Maloney, D. G., Miller, R. A., Thielemans, K., Warnke, R. and Levy, R. A clinical trial of anti-idiotype therapy for B cell malignancy. *Blood*, **65**, 1349–1363 (1985).
19) Miller, R. A., Lowder, J., Meeker, T. C., Brown, S. and Levy, R. Anti-idiotypes in B-cell tumor therapy. *Natl. Cancer Inst. Monogr.*, **3**, 131–134 (1987).
20) Hamada, H., Miura, K., Ariyoshi, K., Heike, Y., Sato, S., Kameyama, K., Kurosawa, Y. and Tsuruo, T. Mouse-human chimeric antibody against the multidrug transporter P-glycoprotein. *Cancer Res.*, **50**, 3167–3171 (1990).
21) Marx, J. L. Antibodies made to order: chimeric antibodies — which are part mouse and part human — may help solve the problems hindering the therapeutic use of monoclonal antibodies. *Science*, **229**, 455–456 (1985).
22) Morrison, S. L. and Oi, V. T. Genetically engineered antibody molecules. *Adv. Immunol.*, **44**, 65–92 (1989).
23) Short, J. M., Fernandez, J. M., Sorge, J. A. and Huse, W. D. λZAP: a bacteriophage λ expression vector with *in vivo* excision properties. *Nucleic Acids Res.*, **16**, 7583–7600 (1988).
24) Frischauf, A-M., Lehrach, H., Poustka, A. and Murray, N. Lambda replacement vectors carrying polylinker sequences. *J. Mol. Biol.*, **170**, 827–842 (1983).
25) Kameyama, K., Imai, K., Itoh, T., Taniguchi, M., Miura, K. and Kurosawa, Y. Convenient plasmid vectors for construction of chimeric mouse/human antibodies. *FEBS Lett.*, **244**, 301–306 (1989).
26) 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).
27) Hamada, H., Maezawa, K. and Tsuruo, T. Nucleotide sequences of the variable regions of a mouse monoclonal antibody MRK16. *Nucleic Acids Res.*, **18**, 1900 (1990).
28) Gubler, U. and Hoffman, B. J. A simple and very efficient method for generating cDNA libraries. *Gene*, **25**, 263–269 (1983).
29) Benton, W. D. and Davis, R. W. Screening λgt recombinant clones by hybridization to single plaques *in situ*. *Science*, **196**, 180–182 (1977).
30) Maniatis, T., Fritsch, E. F. and Sambrook, J. "Molecular Cloning: A Laboratory Manual" (1982). Cold Spring Harbor Laboratory, New York.
31) 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).
32) Toneguzzo, F., Hayday, A. C. and Keating, A. Electric field-mediated DNA transfer: transient and stable gene expression in human and mouse lymphoid cells. *Mol. Cell. Biol.*, **6**, 703–706 (1986).
33) Underwood, P. A. Removal of bovine immunoglobulin from serum in hybridoma culture media using protein A. *Methods Enzymol.*, **121**, 301–306 (1986).
34) Goding, J. W. "Monoclonal Antibodies: Principles and Practice" (1983). Academic Press, New York.
35) Hamada, H. and Tsuruo, T. Determination of membrane antigens by a covalent crosslinking method with monoclonal antibodies. *Anal. Biochem.*, **160**, 483–488 (1987).
36) 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).
37) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685 (1970).
38) Hamada, H. and Tsuruo, T. Growth inhibiton of multidrug-resistant cells by monoclonal antibodies against P-glycoprotein. *In* "Molecular and Cellular Biology of Multidrug Resistance in Tumor Cells," ed. I. B. Roninson, pp. 373–391 (1991). Plenum Publishing Corp., New York.
39) Brüggemann, M., Williams, G. T., Bindon, C. I., Clark, M. R., Walker, M. R., Jefferis, R., Waldmann, H. and Neuberger, M. S. Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies. *J. Exp. Med.*, **166**, 1351–1361 (1987).
40) Steplewski, Z., Sun, L. K., 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).
41) 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).
42) Knox, S. J., Levy, R., Hodgkinson, S., Bell, R., Brown, S., Wood, G. S., Hoppe, R., Abel, E. A., Steinman, L., Berger, R. G., Gaiser, C., Young, G., Bindl, J., Hanham, A. and Reichert, T. Observations on the effect of chimeric anti-CD4 monoclonal antibody in patients with mycosis fungoides. *Blood*, **77**, 20–30 (1991).
43) Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M. M., Pastan, I. and Willingham, M. C, Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl. Acad. Sci. USA*, **84**, 7735–7738 (1987).
44) Sugawara, I., Nakahama, M., Hamada, H., Tsuruo, T. and Mori, S. Apparent stronger expression in the human adrenal cortex than in the human adrenal medulla of *Mr* 170,000–180,000 P-glycoprotein. *Cancer Res.*, **48**, 4611–4614 (1988).
45) Cordon-Cardo, C., O'Brien, J. P., Casals, D., Rittman-Grauer, L., Biedler, J. L., Melamed, M. R. and Bertino, J. R. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc. Natl. Acad. Sci. USA*, **86**, 695–698 (1989).
46) Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M. M., Pastan, I., and Willingham, M. C. Immunohistochemical localization in normal tissues of different epitopes in the multidrug transport protein P170: evidence for localization in brain capillaries and crossreactivity of one antibody with a muscle protein. *J. Histochem. Cytochem.*, **37**, 159–164 (1989).
47) Galski, H., Sullivan, M., Willingham, M. C., Chin, K., Gottesman, M. M., Pastan, I. and Merlino, G. T. Expression of a human multidrug resistance cDNA (*MDR1*) in the bone marrow of transgenic mice: resistance to daunomycin-induced leukopenia. *Mol. Cell. Biol.*, **9**, 4357–4363 (1989).
48) Mickisch, G. H., Merlino, G. T., Galski, H., Gottesman, M. M. and Pastan, I. Transgenic mice that express the human multidrug-resistance gene in bone marrow enable a rapid identification of agents that reverse drug resistance. *Proc. Natl. Acad. Sci. USA*, **88**, 547–551 (1991).
49) Riechmann, L., Clark, M., Waldmann, H. and Winter, G. Reshaping human antibodies for therapy. *Nature*, **332**, 323–327(1988).
50) Queen, C., Schneider, W. P., Selick, H. E., Payne, P. W., Landolfi, N. F., Duncan, J. F., Avdalovic, N. M., Levitt, M., Junghans, R. P. and Waldmann, T. A. A humanized antibody that binds to the interleukin 2 receptor. *Proc. Natl. Acad. Sci. USA*, **86**, 10029–10033 (1989).
51) Co, M. S., Deschamps, M., Whitley, R. J. and Queen, C. Humanized antibodies for antiviral therapy. *Proc. Natl. Acad. Sci. USA*, **88**, 2869–2873 (1991).
52) Gorman, S. D., Clark, M. R., Routledge, E. G., Cobbold, S. P. and Waldman, H. Reshaping a therapeutic CD4 antibody. *Proc. Natl. Acad. Sci. USA*, **88**, 4181–4185 (1991).