Jpn. J. Cancer Res. 80, 1006–1013, October 1989

Multidrug Resistance in Cultured Human Leukemia and Lymphoma Cell Lines Detected by a Monoclonal Antibody, MRK16

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Forty cultured human leukemia and lymphoma cell lines never exposed to anticancer agents in culture, apart from doxorubicin (ADM)-resistant K562/ADM, were examined for reactivity with a monoclonal antibody, MRK16 in $F(ab')_2$ form [MRK16-$F(ab')_2$], which recognizes P-glycoprotein (P-gp). The relative resistance index to various drugs was calculated by dividing the 50% growth inhibitory concentration (IC_{50}) of the test cell line by IC_{50} of K562, which was the negative control in the antibody experiment. MRK16-$F(ab')_2$ reacted with four cell lines, K562/ADM, KYO-1, HEL and CMK, which had relative resistance index values of 2 or more to vincristine (VCR), vindesine, vinblastine, ADM, daunorubicin, mitoxantrone (MIT), etoposide (VP-16) and actinomycin-D (ACT-D). The level of resistance to VCR and ADM in these cell lines decreased significantly in the presence of 10 μM verapamil *in vitro*. Significant expression of mRNA of P-gp gene was also detected in K562/ADM, KYO-1 and HEL. MRK16-$F(ab')_2$ did not react with 36 other cell lines. Among them, three cell lines, PL-21, P31/FUJ and KOPM-28, had relative resistance index values of 2 or more to anthracyclines, MIT and VP-16, but not to *vinca* alkaloids or ACT-D. The level of ADM-resistance in these cell lines did not decrease significantly in the presence of 10 μM verapamil. Five cell lines, ATL-1K, HL-60, KMOE-2, ML-1 and U266, had relative resistance index values of 2 or more to some of the drugs, but not to the others, and 19 other cell lines did not. These results indicate that the reactivity of MRK16-$F(ab')_2$ correlates with a relative resistance index of 2 or more to all these drugs in cultured human leukemia and lymphoma cell lines.

Key words: Multidrug resistance — Monoclonal antibody — Leukemia — Lymphoma — Culture cell

Drug resistance is a major reason for treatment failure of hematologic malignancies resulting in fatality. Detection of drug-resistant human tumor cells still presents difficult problems that remain to be solved. Among various types of drug resistance, multidrug resistance (MDR)[1-3] is currently receiving most attention. The cultured MDR cell lines induced *in vitro* often show cross-resistance to structurally unrelated drugs owing to the lowered accumulation and enhanced efflux of certain anticancer agents by the cells.[4,5] This change in drug transport in the MDR cells correlates with increased expression of a membrane glycoprotein with a molecular mass of 170 to 180 kilodaltons (P-glycoprotein, P-gp).[3,6] P-gp-associated MDR can be reversed efficiently by calcium antagonists.[7,8]

Very recently, *mdr* 1 or P-gp genes corresponding to P-gp have been isolated.[9-12] By using complementary DNA (cDNA) clones of P-gp gene, the expression of the P-gp messenger RNA (mRNA) has been demonstrated in normal colon, kidney, liver and adrenal gland of humans at readily detectable levels, but not in other tissues, including lymphatic and hematopoietic organs.[13] Several human tumor cell lines of kidney origin were shown to be intrinsically resistant to drugs, partly because of the expression of P-gp.[14]

Recently a monoclonal antibody recognizing P-gp, MRK16, was established.[15] MRK16 reacted with doxorubicin (ADM)-resistant K562/ADM cells that were induced from K562 cells by ADM *in vitro*, but not with the parent K562 cells derived from chronic myelogenous leukemia in blastic crisis (CMLbc). We conducted experiments to see if P-gp could occur in cultured human leukemia and lymphoma cell lines without selection by anticancer agents *in vitro*. In order to avoid false-positive reaction of the antibody through a receptor specific for a crystallizable fragment of IgG in the cell surface, the antibody-binding fragment of MRK16 digested by pepsin [MRK16-$F(ab')_2$] was used in the experiment. We found that MRK16-$F(ab')_2$ reacted with three cell lines. They seemed to be typical MDR cells in terms of cross-resistance pattern, significant reversal of MDR by verapamil (VER) and sig-

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nificant expression of mRNA of P-gp gene. The results indicate that MRK16-F(ab′)$_2$ can detect typical MDR cells.

MATERIALS AND METHODS

Monoclonal antibody The anti-P-gp monoclonal antibody, MRK16, was obtained as described previously[15] by immunizing BALB/c mice with intact K562/ADM cells.[3] MRK16 was selected as a monoclonal antibody that reacted with K562/ADM, but not with the parent cell line, K562. The immunoglobulin (Ig) type of MRK16 is IgG_{2a}. MRK16-F(ab′)$_2$ was prepared as described elsewhere.[16] In this study, MRK16-F(ab′)$_2$ was always used in order to avoid nonspecific false-positive reactions through a receptor specific for a crystallizable fragment of IgG in the cell surface.

Cell lines Forty human cultured cell lines (eight T-cell, five non-T, non-B cell, ten B-cell and 17 myeloid cell lines as listed in Table I) derived from leukemias and lymphomas were used in the present study. The cells were cultured in a growth medium, RPMI1640 (GIBCO, Long Island, N.Y.) supplemented with 10% fetal calf serum, 100 μg/ml aminobenzyl penicillin and 20 μg/ml gentamicin, in a floating state at 37°C under a humidified atmosphere in a 5% CO_2 incubator (Forma Scientific Ltd., Marietta, Ohio). All cell lines except K562/ADM had never been exposed to any anticancer agent. K562/ADM had been maintained in the presence of 300 ng/ml ADM. These cells were used for experiments in the exponentially proliferating phase.

Indirect immunofluorescence assay Cultured cells were analyzed for reactivity of MRK16-F(ab′)$_2$ by an indirect immunofluorescence method using an Ortho Spectrum III laser flow cytometry system (Ortho Diagnostic Systems Inc. Westwood, Mass.). NS-1 myeloma ascites[28] was used as a negative control antibody for background fluorescence. K562/ADM cells were used as a positive control and K562 as negative control for MRK16-F(ab′)$_2$. All reagents were titrated for the positive control to obtain optimum reactivity and used in titrated excess to avoid false-negative reaction. In brief, 0.5 to 1×10^5 cells in a Fisher tube (Laboscience Co., Ltd., Tokyo; Cat. No. J4-978-145) were incubated with 15 μl of MRK16-F(ab′)$_2$ (105 μg/ml) or negative control antibody at 4°C for 30 min. The cells were washed three times, and incubated with 15 μl of a 1/200 dilution of fluorescein-conjugated F(ab′)$_2$ fragments of sheep anti-mouse IgG (Silenus Laboratories, Hawthorn, Australia; Batch 339.1) at 4°C for 30 min. Then the cells were washed three times, and the fluorescence staining was recorded on the Ortho Spectrum-III, which displayed the results of the analysis on a histogram. Thresholds were arbitrarily set so that positive cells for the negative control antibody would be less than 4%. Cells demonstrating fluorescence intensity above each threshold were determined as positive using a Data Handling System, Model 2140 (Ortho Diagnostic Systems, Inc.).

Drug sensitivity The sensitivity of 31 representative cell lines (Table II) to each drug was measured by determining the 50% growth-inhibitory concentration (IC_{50}).[7] The cells were suspended in fresh growth medium in a glass test tube (LT-15100, Telmo Co., Ltd., Tokyo) or a 24-well dish (Corning Glass Works, Corning, N.Y.) at an appropriate concentration (about $0.5–1.5\times10^5$ cells/ml) according to the growth rate and cell density required for exponential growth in each cell line throughout the experiment. They were treated with graded concentrations of vincristine (VCR), vindesine (VDS), vinblastine (VLB) (Shionogi & Co., Ltd., Osaka), ADM (Kyowa Hakko Kogyo, Co., Ltd., Tokyo), daunorubicin (DNR; Meiji Seika Kaisha, Ltd., Tokyo), mitoxantrone [MIT; Lederle (Japan), Ltd., Tokyo], etoposide (VP-16; Nippon Kayaku, Co., Ltd., Tokyo) and actinomycin-D (ACT-D; Banyu Pharmaceutical Co., Ltd., Tokyo). The cells were cultured in the presence of the drug for 72 h, and then the cell number was determined with a Coulter Multisizer (Coulter Electronics Limited, Luton, England) as described previously.[19] The growth inhibition of the treated cells was expressed as percentage of that of control cells, where the initial cell numbers were subtracted in the calculation. The relative resistance index of each cell line to the drugs was calculated by dividing the IC_{50} of the test cell line by that of the parental K562 cell line, which was a negative control for the MRK16-antibody experiment. All experiments were performed in duplicate.

Reversal of resistance by VER Nineteen cell lines were selected to observe the degree of reversal of the drug resistance by VER. They were treated with graded drug concentrations of 0.1 to 2,000 n*M* for VCR or 1.0 to 2,000 n*M* for ADM in the absence or presence of 10 μM VER. The degree of reversal of resistance to VCR or ADM by VER was expressed as the value obtained by dividing the IC_{50} value in the absence of VER by that in the presence of VER. The cells treated with VER alone served as one control to determine if VER itself had a growth-inhibitory effect on the cultured cells.

RNA blot analysis Total cellular RNA was extracted according to the method described elsewhere.[29] RNA blot analysis was carried out as described previously.[10, 30] Briefly, RNA samples (10 ng per well) were run on a 1% agarose gel in MOPS buffer [40 m*M* 3-(N-morpholino)-propanesulfonic acid, 10 m*M* sodium acetate, and 1 m*M* ethylenediaminetetraacetic acid, pH 7.0] containing 13.4% formaldehyde, and then transferred to a nitrocellulose filter. The filter was dried and baked at 80°C for 2 h.

The *mdr* 1 cDNA probe in this study was cloned by Sugimoto *et al.*[12] and encodes the 3′-part of P-gp mRNA (1.2 kb). The cDNA probe was labeled to give a specific activity of 10^9 cpm/μg by oligolabeling[31] using [^{32}P]-deoxycytidine 5′-triphosphate (3,000 Ci/mmol). The baked filters were incubated for 6 h at 65°C in 10× Denhardt's solution (1×Denhardt's solution = 0.2 g Ficoll-400, 0.2 g polyvinylpyrrolidone, and 0.2 g bovine serum albumin per liter) containing 0.5% sodium dodecyl sulfate (SDS), then hybridized for 16 h at 65°C in the hybridization mixture containing 4×standard saline citrate (SSC) (1×SSC = 0.15 *M* NaCl, 0.015 *M* sodium citrate pH 7.2), 5×Denhardt's solution, 0.5% SDS, 100 μg/ml sonicated and denatured *E. coli* DNA, 40 μg/ml poly A and 10^7 cpm of ^{32}P-labeled *mdr* 1 cDNA probe. The filter was washed three times for 30 min each at 65°C in 2×SSC and twice at 65°C in 0.2×SSC, 0.1% SDS, then autoradiographed at −70°C on a Kodak XAR-5 film with an intensifying screen.

Statistical analysis The differences between relative resistance indices of test cell lines were evaluated statistically using Student's *t* test. A probability value less than 0.05 was considered statistically significant.

RESULTS

Reactivity of MRK16-F(ab′)$_2$ with various cultured cell lines The percentage of cells positive for MRK16-F(ab′)$_2$ is shown in Table I. MRK16-F(ab′)$_2$ clearly reacted with four cell lines, HEL derived from erythroleukemia, KYO-1 from CMLbc, CMK from megakaryoblastic leukemia and K562/ADM, but not with 36 other cell lines. Figure 1 shows representative fluorescent cytograms of the four positive cell lines. Mean fluorescence intensities of the three cell lines never exposed to drugs in culture were much less than that of K562/ADM, suggesting that their P-gp expression is much less than that of K562/ADM.

Drug sensitivity The relative resistance index values of 31 cell lines against three *vinca* alkaloids (VCR, VDS and VLB), two anthracyclines (ADM and DNR), MIT, VP-16, and ACT-D are shown in Table II. All the cell lines except K562/ADM had never been exposed to any anticancer agent *in vitro*. Nevertheless, some cell lines were resistant to the drugs, as compared to K562 (negative control in the antibody experiment). Among these cell lines, three cell lines, KYO-1, HEL and CMK, had relative resistance index values of 2 or more against all these drugs, indicating that the three cell lines were more resistant to *vinca* alkaloids (VCR, VDS and VLB) ($P<0.01$), anthracyclines (ADM and DNR), MIT, VP-16 ($P<0.001$) and ACT-D ($P<0.01$) than K562. Two cell lines, ATL-1K derived from adult T-cell leukemia and HL60 from acute promyelocytic leukemia (APL), also

Table I. Reactivity of MRK16-F(ab′)$_2$ with Cultured Cell Lines

Cell line[a]	Origin	MRK16-F(ab′)$_2$ (positive percent)[b]
T-cell lines		
RPMI-8402	ALL	0
HPB-ALL	ALL	0
P12/ICH	ALL	0
MOLT3	ALL	0
MOLT4B	ALL	0
SKW3	CLL	0
HUT-102	ATL	0
ATL-1K	ATL	0
Non-T, non-B cell lines		
Reh	ALL	0
KM-3	ALL	0
NALM-6	ALL	0
KOPN-1	ALL	0
P30/OHK	ALL	0
B-cell lines		
P3HR-1	Burkitt	0
Daudi	Burkitt	0
Raji	Burkitt	0
P32/ISH	Burkitt	0
OHNO	NHL	0
A5/TAK	NHL	0
A4/FUK	NHL	0
BALL-1	ALL	0
RPMI-8226	MM	0
U266	MM	0
Myeloid cell lines		
K562	CMLbc	0
K562/ADM	CMLbc	**100**
KG-1	AML	1
ML-1	AML	1
HL-60	APL	0
PL-21	APL	2
P31/FUJ	AMoL	0
THP-1-O	AMoL	1
P39/TSU	AMMoL	1
EoL-E5	EoL	0
KMOE-2	ErL	4
HEL	ErL	**62**
KCL-22	CMLbc	2
KOPM-28	CMLbc	4
KYO-1	CMLbc	**74**
CMK	AMKL	**41**
U937	DHL	1

ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; ATL, adult T-cell leukemia; Burkitt, Burkitt's lymphoma; NHL, non-Hodgkin's lymphoma; MM, multiple myeloma; AML, acute myelogenous leukemia; AMoL, acute monocytic leukemia; AMMoL, acute myelomonocytic leukemia; EoL, eosinophilic leukemia; ErL, erythroleukemia; AMKL, acute megakaryoblastic leukemia; DHL, diffuse histiocytic lymphoma.

a) Original papers for the cell lines described above were listed in the previous reports[3,17-21] except PL-21,[22] EoL-E5,[23] KMOE-2,[24] KCL-22,[25] KOPM-28[26] and CMK.[27]

b) Bold-faced data indicate positive reactivity with the antibody, and the percentage of positive cells is given.

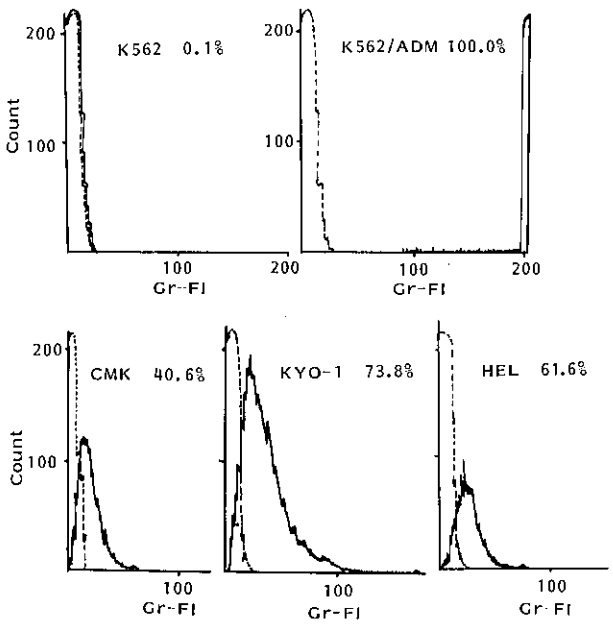

Fig. 1. Flow cell histograms of K562, K562/ADM, CMK, KYO-1 and HEL treated with MRK16-F(ab′)$_2$. ——, MRK16-F(ab′)$_2$; ·····, control antibody; Gr-Fl, relative green fluorescence intensity. Cultured cells were analyzed by an indirect immunofluorescence method using an Ortho Spectrum III laser flow cytometry system. The fluorescence staining was recorded as a histogram (shown in this figure). Thresholds were arbitrarily set so that positive cells for the negative control would be less than 4%. Cells demonstrating fluorescence intensity above each threshold were determined as positive. The percentage of positive cells for MRK16-F(ab′)$_2$ is shown in the figure.

had relative resistance index values of 2 or more against some of the drugs such as VCR, ADM, MIT and VP-16 for ATL-1K, and VCR, VP-16 and ACT-D for HL-60. The results indicate that the two cell lines are more resistant than K562 to these drugs ($P<0.05$), but not to the others. In the same way, three other cell lines, PL-21 derived from APL, P31/FUJ from acute monocytic leukemia and KOPM-28 from CMLbc, appeared to be more resistant than K562 to anthracyclines (ADM and DNR), MIT ($P<0.001$) and VP-16 ($P<0.01$), but not to *vinca* alkaloids or ACT-D. In addition to these cell lines, there were some cell lines with isolated resistances. KMOE-2 derived from erythroleukemia was more resistant than K562 to both MIT and ACT-D, and both ML-1 derived from acute myelogenous leukemia and U266 derived from myeloma were more resistant than K562 to ACT-D alone.

Effect of VER on growth-inhibitory activity of VCR and ADM Table III shows that the IC_{50} values for VCR and ADM in four MRK16-F(ab′)$_2$-positive cell lines, K562/ADM, KYO-1, HEL and CMK, decreased more significantly in the presence of 10 μM VER than those of 15 other antibody-negative cell lines. In the case of VCR-resistance, the decrease in the sensitivity level by VER was more striking (18-fold or more decrease) in the antibody-positive cell lines than that in the antibody-negative cell lines (3.5-fold or less decrease) ($P<0.001$). In the case of ADM-resistance, it was slight but still greater (more than 2-fold) in the antibody-positive cell lines than in the antibody-negative cell lines (1.7-fold or less) including the negative control K562 (1.5-fold) ($P<0.05$). Potentiation of drug sensitivity by VER was always greater for VCR than for ADM.

RNA blot analysis The expression of mRNA of the P-gp gene in two MRK16-F(ab′)$_2$-positive cell lines, KYO-1 and HEL, and one antibody-negative cell line, P3HR-1 derived from Burkitt's lymphoma, was examined. K562/ADM was used as a positive control. As shown in Fig. 2, mRNA of the P-gp gene was significantly expressed in KYO-1 and HEL, though the expression in the two cell lines was weaker than that in K562/ADM. The expression of P-gp mRNA could not be detected in P3HR-1.

DISCUSSION

When developing an experimental *in vitro* system to investigate drug resistance, it is important to consider whether or not the system is adequate for studies on human clinical resistance. All the cell lines except K562/ADM had never been exposed to any anticancer agent *in vitro*, although they were derived from patients at relapse or in the refractory stage of leukemia or lymphoma after conventional chemotherapy. Therefore, unlike other experimental systems using highly resistant cultured cells that were selected by stepwise passage in increasing concentrations of drugs, our experimental system should closely reflect clinical resistance.

In this study, we used K562 as an index cell line to evaluate relative resistance. K562 was derived from a patient with CMLbc, which is an intractable disease, and is the parental cell line of K562/ADM, from which we established the anti-P-gp monoclonal antibody, MRK16. We evaluated the reactivity of MRK16-F(ab′)$_2$ with various cultured leukemia and lymphoma cells with the use of K562 as a negative control and K562/ADM as a positive control. Cell lines having a relative resistance index of 2 or more to drugs are significantly more resistant to the drugs than K562, and so this value of relative resistance index was employed as a criterion of drug resistance.

A good correlation between reactivity of MRK16-F(ab′)$_2$ and relative resistance index of 2 or more to three *vinca* alkaloids (VCR, VDS and VLB), two anthracyclines (ADM and DRN), MIT, VP-16 and

Table II. Degree of Relative Resistance

Cell line (lineage)[a]		Relative resistance index[b]							
		VCR	VDS	VLB	ADM	DNR	MIT	VP-16	ACT-D
K562/ADM	(M)	**500**	**970**	**130**	**100**	**80**	**20**	**30**	**130**
KYO-1	(M)	**23**	**80**	**12**	**4.8**	**5.0**	**12**	**12**	**6.2**
HEL	(M)	**16**	**30**	**3.0**	**4.3**	**2.7**	**12**	**6.3**	**3.4**
CMK	(M)	**6.5**	**2.4**	**2.0**	**3.6**	**2.5**	**10**	**2.0**	**3.6**
ATL-1K	(T)	**2.6**	0.2	0.6	**3.0**	1.1	**3.6**	**8.3**	0.8
HL-60	(M)	**2.1**	1.0	0.8	1.3	1.3	0.7	**2.0**	**2.0**
PL-21	(M)	1.0	0.2	0.7	**4.0**	**2.1**	**2.3**	**2.8**	0.2
P31/FUJ	(M)	1.0	0.9	0.7	**3.2**	**2.4**	**2.8**	**3.1**	0.6
KOPM-28	(M)	0.9	0.2	0.7	**2.8**	**2.8**	**4.4**	**6.7**	1.1
KMOE-2	(M)	0.4	0.2	1.3	1.4	1.5	**5.2**	0.8	**10**
ML-1	(M)	1.6	0.2	0.8	0.9	0.6	1.1	0.7	**2.0**
U266	(B)	1.5	0.7	0.6	1.2	1.0	1.5	1.8	**3.4**
P39/TSU	(M)	1.6	0.2	0.3	0.8	0.9	0.8	0.8	1.2
NALM-6	(N)	1.1	0.8	1.2	0.6	0.8	0.8	0.4	0.7
P32/ISH	(B)	0.8	0.3	0.7	0.6	0.4	0.1	0.7	0.1
Raji	(B)	0.8	1.0	0.9	0.3	0.5	0.1	0.1	0.9
P30/OHK	(N)	0.6	0.3	0.4	1.4	0.8	0.1	1.5	0.5
THP1-O	(M)	0.5	0.6	0.8	1.7	0.6	0.3	1.0	0.8
KM-3	(N)	0.5	0.3	0.9	0.2	0.3	0.1	1.2	0.4
Daudi	(B)	0.5	0.7	0.5	0.2	0.4	0.1	0.1	0.3
RPMI-8402	(T)	0.4	0.5	0.3	0.5	0.6	0.1	0.6	1.6
MOLT-3	(T)	0.4	0.2	0.3	0.6	0.6	1.2	0.4	0.3
P12/ICH	(T)	0.3	0.6	0.6	0.6	0.9	0.3	0.6	0.7
MOLT4B	(T)	0.3	0.3	0.2	1.9	1.1	0.8	1.4	0.4
HPB-ALL	(T)	0.3	0.3	0.3	0.9	0.6	0.3	1.6	0.4
BALL-1	(B)	0.3	0.3	0.9	1.6	0.8	1.5	1.4	0.6
EOL-E5	(M)	0.3	0.7	0.5	0.4	0.6	1.3	0.3	0.4
Reh	(N)	0.3	0.3	0.9	0.4	0.3	0.1	0.6	0.5
A5/TAK	(B)	0.3	0.1	0.3	0.4	0.3	1.0	0.9	0.3
P3HR-1	(B)	0.3	0.3	0.9	0.3	0.4	0.8	0.3	1.2
K562	(M)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
IC_{50} of K562	(n*M*)	1.7	1.7	2.8	12	9.9	3.5	59	1.6

The 50% growth-inhibitory concentration (IC_{50}) of each cell line to each drug was determined by conventional growth inhibition assay. The relative resistance index of each cell line to the drugs was calculated by dividing the IC_{50} of the test cell line by that of the K562 cell line (last row in the table). Bold-faced data indicate relative resistance index values of 2.0 or more. Cell lines having the relative resistance index values of 2.0 or more to drugs were significantly more resistant to the drugs than K562 ($P<0.05$).

a) M, Myeloid; T, T-cell; B, B-cell; N, non-T,non-B.

b) Relative resistance index was calculated by dividing the IC_{50} value of the test cell line by that of the control K562.

VCR, vincristine; VDS, vindesine; VLB, vinblastine; ADM, doxorubicin; DNR, daunorubicin; MIT, mitoxantrone; VP-16, etoposide; ACT-D, actinomycin-D.

ACT-D was demonstrated in the present study using 40 cultured human leukemia and lymphoma cell lines. The cross-resistance to all these drugs in the four cell lines reactive with MRK16-F(ab′)$_2$ was consistent with that of typical MDR cells.[1-3] The resistance to VCR and ADM in these cell lines could be significantly reversed by a calcium antagonist, VER in much the same way as reported for other MDR cells.[7,8] In addition, a significant amount of mRNA of P-gp gene was expressed in the two antibody-positive cell lines tested (KYO-1 and HEL). The results indicate that these antibody-positive cell lines were typical P-gp-associated MDR cells.

Table III. Effect of 10 μM Verapamil (VER) on the Growth-inhibitory Activity of Vincristine (VCR) and Doxorubicin (ADM)

Cell line	VCR IC_{50} (nM)		Fold decrease[a]	ADM IC_{50} (nM)		Fold decrease[a]
	VER(−)	VER(+)		VER(−)	VER(+)	
K562/ADM	1100	23	48	1200	73	16
KYO-1	35	1.1	32	100	28	3.6
HEL	29	1.6	18	58	24	2.4
CMK	8.0	0.4	20	38	18	2.1
ATL-1K	3.8	1.1	3.5	13	7.7	1.7
HL-60	3.5	1.3	2.7	18	12	1.5
PL-21	1.9	0.9	2.1	47	38	1.2
P31/FUJ	2.3	1.1	2.1	40	26	1.5
KOPM-28	2.0	0.8	2.5	26	21	1.2
K562	1.7	0.6	2.8	12	7.9	1.5
ML-1	1.5	0.6	2.7	11	7.3	1.5
Reh	0.5	0.2	2.5	6.7	4.4	1.5
P30/OHK	1.0	0.4	2.5	9.3	7.9	1.2
Daudi	0.9	0.3	3.0	6.1	4.3	1.4
P3HR-1	0.4	0.2	2.0	2.6	2.5	1.0
Raji	1.5	0.5	3.0	4.6	3.9	1.2
RPMI-8402	0.6	0.2	3.0	4.0	3.1	1.3
HPB-ALL	0.5	0.2	2.5	14	10	1.4
MOLT4B	0.5	0.2	2.5	16	13	1.2

a) Fold decrease was obtained by dividing the IC_{50} value in the absence of VER by that in the presence of VER. It was used as an indicator of the degree of reversal of resistance by VER. Significant potentiation of drug sensitivity to ADM ($P<0.001$) and VCR ($P<0.05$) by VER was observed in the four antibody-positive cell lines (K562/ADM, KYO-1, HEL and CMK), but not in the other cell lines. Potentiation of drug sensitivity by VER was always greater for VCR than for ADM.

Three antibody-positive cell lines, KYO-1, HEL and CMK, were derived from CMLbc, erythroleukemia and acute megakaryoblastic leukemia, respectively. It is of interest to note that P-gp was expressed in the three cell lines without selection by drugs *in vitro,* and that these cell lines had been derived from diseases known to be clinically resistant to standard chemotherapy.[32-34]

Among antibody-negative cell lines, three cell lines (PL-21, P31/FUJ and KOPM-28) were cross-resistant to anthracyclines, MIT and VP-16, but not to *vinca* alkaloids or ACT-D. In these cell lines, no significant reversal of ADM-resistance by VER was observed. Of note was the result that these cell lines displayed the same cross-resistance pattern as "atypical MDR."[35,36] The results indicate that the "atypical MDR" may not be related to P-gp. Two cell lines, ATL-1K and HL60, showed relatively cross-resistance to VCR alone among *vinca* alkaloids, VP-16 and some other drugs (Table II), but MRK16-F(ab′)$_2$ did not react with the cells. No significant reversal of VCR-resistance by VER in the two cell lines were observed in this study or in another.[37] These results suggest that P-gp (*mdr*1) gene may not be

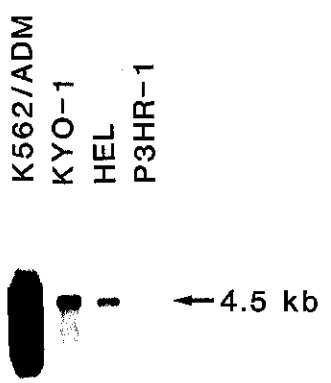

Fig. 2. RNA blot hybridization of K562/ADM, KYO-1, HEL, and P3HR-1 with [^{32}P]P-gp probe. Total cellular RNA (10 μg per well) was run on a 1% agarose gel in MOPS buffer containing 13.4% formaldehyde, and then transferred to a nitrocellulose filter. The baked filters were incubated for 6 h at 65°C in 10×Denhardt's solution containing 0.5% SDS, hybridized in the hybridization mixture containing 10^7 cpm of ^{32}P-labeled *mdr* 1 cDNA probe, and then autoradiographed. The *mdr* 1 mRNA was expressed strongly in K562/ADM and moderately in KYO-1 and HEL, but not in P3HR-1.

involved in the drug resistance of these cell lines. However, there is still the possibility that the cell lines might possess a low but biologically significant level of P-gp that is insufficient to react with MRK16-F(ab′)$_2$ or a different type of P-gp that does not contain the epitope detected by MRK16-F(ab′)$_2$. It is also possible that different mechanism(s) from that involving P-gp might operate in VCR-resistance.[37] To solve these problems, further studies are needed on the sensitivity and specificity of MRK16-F(ab′)$_2$ by comparing MRK16-F(ab′)$_2$ reactivity with expression of P-gp (*mdr*1) mRNA in all the cell lines. Nevertheless, this is the first paper demonstrating a good correlation between reactivity to an anti-P-gp antibody [MRK16-F(ab′)$_2$] and typical MDR in cultured human leukemia and lymphoma cell lines without selection by drugs *in vitro*, suggesting that MRK16-F(ab′)$_2$ may be applicable to detect P-gp-associated MDR cells in clinical samples.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid for Cancer Research (62S-1, 61-2, 62-23) and for the Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare. The authors are grateful to Drs. S. Nakazawa, National Saitama Hospital, for providing KOPN-1 and KOPM-28 cell lines, H. Saito, Tohoku University, for Utsumi and EoL-E5, H. Okano, National Kyushu Cancer Center, for KMOE-2, I. Kubonishi and I. Miyoshi, Kochi Medical School, for BALL-1, KCL-22 and PL-21, T. Sato, Chiba University, for CMK, K. Kita, Mie University, for KYO-1 and J. Minowada, the Fujisaki Cell Center, for the other cell lines except K562/ADM, ATL-1K, P31/FUJ, P39/TSU, P30/OHK, P12/ICH and A5/TAK. The authors also thank Associate Professor J. Patrick Barron of St. Marianna University School of Medicine for comments on the manuscript.

(Received May 29, 1989/Accepted August 3, 1989)

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