Monoclonal Antibody to an External Epitope of the Human mdrl P-Glycoprotein

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ABSTRACT

A membrane glycoprotein, termed P-glycoprotein, has been shown to be responsible for cross-resistance to a broad range of structurally and functionally distinct cytotoxic agents. P-glycoprotein, encoded in humans by the mdrl gene, functions as an energy-dependent efflux pump to exclude these cytotoxic agents from the resistant cell. In order to study the phenomenon of multidrug resistance in both normal and neoplastic cells, we have generated a mouse monoclonal antibody directed to an external epitope of the human P-glycoprotein. This monoclonal antibody, 4E3, is an IgG2a class antibody which specifically recognizes the human mdrl P-glycoprotein but not the mdrl gene product. The 4E3 monoclonal antibody immunoprecipitates both the glycosylated and nonglycosylated forms of P-glycoprotein under mild denaturation conditions. In addition, 4E3 can detect P-glycoprotein in immunocytochemical analysis of fixed tissue-cultured cells and in analysis of frozen sections of human tissue. Binding of the monoclonal antibody to multidrug-resistant cells does not significantly affect the intracellular accumulation or potentiate the cytotoxicity of daunomycin in multidrug-resistant cells. However, at high concentrations of antibody, 4E3 produces a mild potentiation of vinblastine and actinomycin cytoxicity in multidrug-resistant cells. This monoclonal antibody will be useful both for analyzing P-glycoprotein expression in normal and neoplastic cells and for isolating live cells expressing the P-glycoprotein without significantly affecting the efflux functions of the transporter.

INTRODUCTION

Resistance of malignant tumors to chemotherapeutic agents remains the major cause of failure in cancer therapy. The analysis of cell lines displaying resistance to multiple drugs in vitro has provided significant insight into the mechanisms of multidrug resistance. A multigene family, designated mdrl, has been identified, the members of which encode a family of membrane glycoproteins termed P-glycoproteins (1–5). The mdrl gene is overexpressed in multidrug-resistant cell lines which, although selected for resistance to a single cytotoxic agent, display cross-resistance to a broad spectrum of structurally and functionally unrelated compounds. The compounds which comprise the multidrug resistance phenotype include many of the most potent natural product agents currently used in cancer chemotherapy. i.e., the anthracyclines, Vinca alkaloids, epipodophyllotoxins, and certain protein synthesis inhibitors such as actinomycin D (1–5). The mdrl P-glycoprotein appears to function as an energy-dependent transport pump capable of effluxing cytotoxic agents and thereby decreasing their intracellular concentration (6, 7).

The mammalian mdrl multigene family is differentially expressed in both normal and malignant tissues (8–12). The highest levels of mdrl expression are found in the adrenal gland, a variety of specialized secretory epithelial surfaces, and the capillary endothelium of blood vessels in the brain, skin, and testes (8, 13–17). Tumors which express the highest levels of P-glycoprotein are often derived from tissues which also express high levels of this gene product (8, 9, 11, 12). Several recent studies have indicated that the response to therapy may be correlated with the level of expression of P-glycoprotein (18–28), and increased levels of P-glycoprotein expression have occasionally been observed in malignancies following exposure to chemotherapeutic drugs (8, 9, 11, 12). A wide variety of compounds have been identified which are capable of reversing the multidrug-resistant phenotype in drug-resistant cells, suggesting that the level of P-glycoprotein expression not only may predict the response of individual tumors to specific cytotoxic agents but also may provide important criteria for determining successful chemotherapeutic regimens (29, 30).

In an effort to develop a sensitive reagent which detects P-glycoprotein expression, we have generated a new monoclonal antibody, 4E3, which recognizes an external epitope of the human mdrl P-glycoprotein. This monoclonal antibody is effective at immunoprecipitation and immunocytochemical detection of P-glycoprotein. It is able to recognize both the nonglycosylated and glycosylated forms of P-glycoprotein. Functional characteristics of its reactivity distinguish this monoclonal antibody from the others described in the literature and provide support for its usefulness in characterizing and isolating cells which express the P-glycoprotein.

MATERIALS AND METHODS

Cell Lines. The human T-cell lymphoblastic leukemia cell line CEM and its multidrug-resistant derivative CEM/VBL 100 (maintained in 100 ng/ml vinblastine) were kindly provided by Dr. William Beck (St. Jude’s Hospital for Sick Children, Memphis, TN). The CEM/VBL 100 cell line was placed in increasing concentrations of vinblastine to generate the CEM/VBL 300 and CEM/VBL 500 lines, which are maintained in 250 ng/ml and 500 ng/ml vinblastine, respectively. ME180/Dox 500 is a multidrug-resistant cell line derived by stepwise selection of the human uterine adenocarcinoma cell line ME180 (American Type Culture Collection) in increasing amounts of doxorubicin to a final concentration of 500 ng/ml.4 SW-1573/500 and SW-1573 are multidrug-resistant and drug-sensitive human squamous lung carcinoma cell lines, respectively (31). The multidrug-resistant cell line (An Alexander Adria/0.5 is a human hematopoietic cell line derived by stepwise selection of the drug-sensitive parent in increasing concentrations of doxorubicin to a final concentration of 500 ng/ml (32). The mdrl-transfected cell lines TMDR1 and TMDR3.35 were derived by transfection of the human melanoma cell line BRO with full-length mdrl and mdrl cDNAs (33, 34). Both TMDR1 and TMDR3.35 transfectants showed increased levels of the respective P-glycoproteins, based upon immunohistochemical staining with C219 (data not shown).

Generation of Anti-P-Glycoprotein Monoclonal Antibody. SJL female mice (The Jackson Laboratory, Bar Harbor, ME) were immunized with 12 i.p. injections every 1–2 weeks of 5–10 × 106 SW-1573/500 or ME180/Dox 500 cells. Following these injections, a single i.v. dose of 1 × 10⁷ SW-1573/500 cells was given, and 3 days later the mouse was sacrificed and the splenocytes were harvested. The SJL-derived splenocytes were fused with logarithmically growing P3/NS-1 myeloma cells (American Type Culture Collection) at a 1:3 ratio, using polyethylene glycol (Boeringher-Mannheim), and were then plated with a 1:1 mixture of BALB/c myelomas and splenocytes, which served as a feeder layer. The cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 20% FBS, 1 mM hypoxanthine, 4 μM aminopterin, 0.16 μM thymidine, and 5% “1st Endo” conditioned medium. The 1st Endo conditioned medium is derived from a mouse embryonic cell line which has stimulatory activity for B lymphocyte antibody synthesis.4 Eleven days later, the supernatants of approximately 3000 hypoxanthine/aminopterin/thymidine-resistant hybridomas were screened for immunofluorescent staining by flow cytometry to

4 R. J. Arceci, unpublished observations.

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3 The abbreviations used are: MDR, multidrug resistance (resistant); cDNA, complementary DNA; FBS, fetal bovine serum; TBS, Tris-buffered saline; SDS, sodium dodecyl sulfate; Mab, monoclonal antibody; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate; PBS, phosphate-buffered saline; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium.
identify antibodies which would recognize surface epitopes on multidrug-resistant CEM/VBL 500 cells and not on the CEM parental cell line.

Flow Cytometry. Cells were washed twice in cold PBS, resuspended at $1 \times 10^6$ cells in 100 μl of human serum diluted 1:1 in PBS, and then incubated at 4°C for 30 min to block Fc receptors. Two ml of PBS were then added to the cells, which were collected by centrifugation. Pelleted cells were resuspended in 100 μl of PBS containing 2% goat serum and 10 μg/ml 4E3 or a mouse IgG2a isotype-matched control antibody. This mixture was incubated for 30 min at 4°C and cells were washed twice with cold PBS, followed by resuspension in 100 μl of PBS containing 2% goat serum and fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin (Fab)2 fragment (TAGO) at a 1:30 dilution. Cells were incubated with the second antibody for 30 min at 4°C in the dark, followed by two washes in cold PBS and fixation in 2% paraformaldehyde prior to analysis. The level of fluorescence was determined using a Becton-Dickinson FACSCANTO-II and the LYSIS software application.

Immunohistochemistry. Adherent cell lines were grown on polylysine-coated Labtech slide chambers or on coverslips, to approximately 60% confluence. Slides were washed in PBS and fixed for 5 min at 4°C in 1% or 4% paraformaldehyde in PBS. The cells were blocked for 30 min in 0.05 M Tris-buffered saline (pH 7.6) containing 20% horse serum. Liquid was drained from the slides, and they were then incubated for 1 h at room temperature, in humidified boxes, with the primary or control antibodies at 20 μg/ml in TBS containing 1% goat serum. After washing 3 times in TBS, the slides were incubated for 1 h at room temperature with an alkaline phosphatase-conjugated goat anti-mouse secondary antibody (Promega) diluted 1:500. The unbound secondary antibody was washed away with TBS and the slides were dried using a Fast Red substrate, followed by counterstaining in hematoxylin. Frozen sections (5 μm) of cryopreserved human tissues were fixed briefly in acetone at −20°C, followed by binding of the primary or control antibodies at 20 μg/ml. Detection of primary antibody binding was done using the streptavidin-biotin peroxidase method as described by Coggi et al. (35).

Immunoprecipitation. Immunoprecipitations were done under both mild (CHAPS) and stringent (SDS/Triton X-100) denaturation conditions. Cells were metabolically labeled with [35S]methionine (DuPont) at 25–50 μCi/ml overnight or at 100 μCi/ml for 0.5–3 h at 37°C in 5% CO2. At the end of the labeling period, cells were collected and washed twice in cold PBS. For immunoprecipitation under mild denaturation conditions, 5 × 105 cells were lysed in 200 μl of 50 mM Tris-HCl, pH 8.0, 140 mM NaCl, 2 mM MgCl2, 0.5% CHAPS, 1 mM phenylmethylsulfonyl fluoride, 0.2 units/ml aprotinin, 0.02% sodium azide, 10 μM ATP. After shaking for 30 min at 4°C, insoluble material was pelleted by centrifugation at 14,000 × g in a microcentrifuge. The supernatants were transferred to fresh tubes and 50 μl of Protein G-Sepharose beads (Pharmacia), precoated with goat anti-mouse IgG and bovine serum albumin, were added and rocked at 4°C for 1 h to preclay the extracts. The beads were then pelleted for 5 s in a microfuge and primary antibodies were added at 20 μg/ml. The supernatants and antibodies were rocked at 4°C for 2 h, and then 30 μl of Protein G-Sepharose beads were added for an additional 1 h. The beads were then collected by centrifugation and washed 3 times in 50 mM Tris-HCl, pH 8.0, 140 mM NaCl, 2 mM MgCl2, 0.1% CHAPS, 1 mM phenylmethylsulfonyl fluoride, 0.1% bovine hemoglobin, 0.02% sodium azide, followed by three washes in the same buffer without CHAPS or bovine hemoglobin. The beads were then resuspended in 50 μl of sample buffer, incubated at room temperature, and pelleted by centrifugation, and supernatants were electrofocused on a 7.5–15% gradient polyacrylamide gel (36). Immunoprecipitation using SDS/Triton X-100 was performed essentially as described previously (37).

Western Blot Analysis. Immunoprecipitated complexes of metabolically labeled proteins from MDR and parental cell lines were electrophoretically separated as described above and then transferred to nitrocellulose prior to blotting with either the anti-P-glycoprotein monoclonal antibody C219 (Centocor) or mouse IgG2a isotype control antibody. Western blotting was accomplished using an alkaline phosphatase detection system, as described by the manufacturer (Promega).

Cytotoxic Drug Accumulation Studies. Intracellular accumulation of cytotoxic agents was performed using modifications of previously described procedures (38, 39). [3H]Daunomycin (specific activity, 1–5 Ci/mmol) was purchased from DuPont and [3H]vinblastine (specific activity, 5–25 Ci/mmol) from Amersham. Cells were collected, washed 3 times in PBS, and counted. The cells were resuspended at 1 × 105 cells/ml of RPMI medium containing 10% FBS and 10 μg 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. Aliquots of 0.25 ml were dispensed into 75-mm round-bottomed plastic tubes, and the MDR reversal agent cyclosporin A or antibodies were added. The tubes were incubated in a 37°C shaking water bath for 30 min prior to the addition of the tritiated cytotoxic agent. [3H]Daunomycin and [3H]vinblastine (each at approximately 8 nm) were added with unlabeled daunomycin or vinblastine, respectively (final concentration, 10 μM). The tubes were incubated in the presence of the drug at 37°C for 2 h. At the end of this incubation, the entire contents of each tube were overlayed on a 200-μl cushion of Dow 550 silicone oil (Dow Corning) and mineral oil (4:1) in 1.5-ml Eppendorf tubes. The tubes were centrifuged at 10,000 × g at 4°C for 1 min to separate the cells from drug-containing medium. The medium and oil mixture was removed by aspiration and the cell pellets were solubilized in 1 ml of 1 M NaOH at 60°C overnight. Glacial acetic acid (0.2 ml) was added to each tube and the contents were thoroughly mixed prior to transfer into 10 ml of Biofluor scintillation cocktail (DuPont). Radioactivity was quantitated in a Beckman scintillation...
sensitive cells. Fig. 1, A-C, demonstrates indirect immunofluorescent staining with 4E3 on the surface of CEM (a drug-sensitive cell line) and the MDR derivatives CEM/VBL 300 and CEM/VBL 500, neither of which were used during the immunization process. There was no difference between the level of fluorescence with the IgG2a control antibody and the level of detectable surface expression of the 4E3 epitope on the CEM cell line grown to high cell density (Fig. 1A). In

counter with error value of ≤5%. The “fold increase” in radiolabeled drug accumulation was calculated by dividing the amount of radioactivity in the control tube (i.e., the solvent- or isotype control antibody-containing tube) into the amount of radioactivity in the tubes containing different concentrations of MDR inhibitors or antibodies. Values reported are the average of at least three experiments, with each experiment being done in duplicate.

Growth Inhibition Studies. The inhibition of cell growth was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium dye assay as described (40, 41). Cells were plated in 96-well plates in RPMI containing 10% FBS and were incubated at 37°C for approximately 1 h. MDR inhibitors or antibodies at varying concentrations of cytotoxic agents (daunomycin, vincristine, or actinomycin D) were diluted into the medium and added to the cell suspension (final volume, 200 μl). The cells were incubated at 37°C in 5% CO₂ for 4 days, after which time 50 μl of 2 mg/ml MTT dye (Sigma) in PBS were added to the wells. The plates were incubated for 4 h at 37°C and then centrifuged at 450 × g for 5 min. The supernatant (225 μl) was aspirated and 150 μl of dimethylsulfoxide were added and mixed on a plate shaker for 10 min. The absorbance of the color reaction in each well was determined by absorbance spectroscopy at 550 nm and 490 nm, using a Whittaker EIA model MA 310 plate recorder with control.

RESULTS

Flow Cytometric Analysis of 4E3 Surface Staining. Flow cytometric analysis was performed with 4E3 on live MDR and drug-resistant cells. There were no specific bands identified. Metabolically labeled cellular lysates from multidrug-resistant CEM/VBL300 cells were immunoprecipitated with 4E3 (lane 3) or IgG2a (lane 4) and transferred to nitrocellulose. There was no difference in the level of fluorescence with the IgG2a control antibody and the level of surface expression of the 4E3 epitope on the CEM cell line grown to high cell density (Fig. 1A). In

Fig. 3. Monoclonal antibody 4E3 recognizes the P-glycoprotein. Cellular lysates of metabolically [35S]methionine-labeled cells were immunoprecipitated with 4E3 or an IgG2a isotype-specific control, fractionated on a 7-15% SDS-polyacrylamide gel, and transferred to a nitrocellulose membrane in preparation for Western blotting with C219. A, autoradiogram of metabolically labeled cellular lysates from drug-sensitive CEM cell lines immunoprecipitated with 4E3 (lane 1) or IgG2a (lane 2) and transferred to nitrocellulose. There were no specific bands identified. Metabolically labeled cellular lysates from multidrug-resistant CEM/VBL300 cells were immunoprecipitated with 4E3 (lane 3) or IgG2a (lane 4). A Mr 170,000 doublet is identified by 4E3 but not the control antibody in the multidrug-resistant cells (arrows). B, Western blot, using C219, of the samples shown in A transferred to nitrocellulose. Note that C219 identifies the same doublet immunoprecipitated by monoclonal antibody 4E3 (arrows, lane 3).

Fig. 4. Glycosylation is not required for 4E3 reactivity with the P-glycoprotein. Cellular lysates of metabolically [35S]methionine-labeled CEM (lane 1), CEM/VBL300 (lane 2), or CEM/VBL300 cells treated with tunicamycin to block N-linked glycosylation (lane 3) were immunoprecipitated with 4E3. Upper and lower arrows indicate the glycosylated and nonglycosylated forms of P-glycoprotein, respectively.
contrast, the multidrug-resistant derivatives CEM/VBL 500 and CEM/VBL 300 displayed increased levels of surface staining with the 4E3 monoclonal antibody (Fig. 1, B and C). Immunofluorescent staining with 4E3 on the CEM/VBL 300 cell line shifted the fluorescence intensity by approximately 2.5 log units, while the more highly resistant CEM/VBL 500 shifted the fluorescence intensity approximately 3 log units, compared to the IgG2a control antibody. The level of 4E binding to CEM/VBL 500 reached a plateau at antibody concentrations of 10 μg/ml (data not shown).

Transfectants expressing the human mdr1 and mdr3 cDNAs were stained to determine the ability of 4E3 to distinguish between the P-glycoproteins encoded by mdr1 and mdr3 (Fig. 1, D-F). Mab 4E3
showed no significant binding to either the parent cell line BRO or the cell line overexpressing mdr3 (Fig. 1, D and F, respectively). However, there was significant binding to the cell line overexpressing mdr1 (Fig. 1E). These results indicate that 4E3 binds to the surface of multidrug-resistant cells which express the mdr1 gene product, but not that of mdr3. In addition, the detection of P-glycoprotein expression by flow cytometry using 4E3 is achievable in multidrug-resistant cells transfected with the mdr1 cDNA that display only 2-fold levels of resistance.

**Immunoprecipitation of P-Glycoprotein with 4E3.** Immunoprecipitation of radiolabeled cell lysates from drug-sensitive and -resistant cell lines with 4E3 was performed to identify the molecular species with which the monoclonal antibody reacts. Fig. 2A demonstrates that Mab 4E3 precipitates a Mr 170,000 protein species from the multidrug-resistant cells but not the drug-sensitive parent, using the relatively mild denaturing condition of 0.5% CHAPS. In comparison, the Mab C219 does not precipitate P-glycoprotein very effectively under these conditions (Fig. 2A). When immunoprecipitation is done under more stringent denaturation conditions, i.e., in the presence of SDS and Triton X-100, the binding of Mab 4E3 is completely lost, in contrast to the increased efficiency of C219 in precipitating the Mr 170,000 P-glycoprotein (Fig. 2B).

Definitive confirmation that the P-glycoprotein contains the 4E3 epitope was obtained by Western blot analysis, using C219, on the protein species immunoprecipitated with 4E3. The 4E3 immunoprecipitate from metabolically labeled drug-sensitive CEM and multidrug-resistant CEM/VBL 500 cell lines was fractionated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose paper for Western blot analysis using C219. Fig. 3 shows an autoradiograph of the [35S]methionine-labeled immunoprecipitate transferred to the nitrocellulose. A Mr 170,000 protein is immunoprecipitated with 4E3 from the multidrug-resistant cell line and is absent in the drug-sensitive parental cell line (Fig. 3A). An isotype control IgG2a antibody did not reveal any specific immunoprecipitable proteins in either the MDR or drug-sensitive parental cell line. Fig. 3B shows the result of Western blotting of the immunoprecipitated proteins in Fig. 3A with C219, demonstrating that the Mr 170,000 protein immunoprecipitated with 4E3 is recognized by C219.

The electrophoretic separation shown in Fig. 3 resolves a doublet both with 4E3 and with C219. This doublet most probably represents the fully glycosylated and the nonglycosylated forms of P-glycoprotein. To address this issue directly, metabolically labeled proteins from MDR cells treated with tunicamycin to block N-linked glycosylation were immunoprecipitated. Fig. 4 demonstrates that 4E3 effectively immunoprecipitates both glycosylated (Fig. 4, lane 2) and nonglycosylated P-glycoprotein (Fig. 4, lane 3).

**Immunohistochemistry Using Mab 4E3.** The ability of Mab 4E3 to detect the P-glycoprotein by immunohistochemical analysis was determined. Fig. 5 demonstrates that, after fixation of cells in 4% paraformaldehyde, 4E3 is able to specifically stain the SW-1573/500 (Fig. 5a) and Alexander Adria/0.5 (Fig. 5e) multidrug-resistant cell

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*J. M. Croop and R. J. Arceci, unpublished observations.*
lines, both of which have increased expression of the P-glycoprotein. The corresponding drug-sensitive parental cell lines do not demonstrate any staining with 4E3 (Fig. 5, c and g, respectively). Because these cells were fixed with paraformaldehyde alone, the histochemical signal observed with 4E3 is mostly due to surface staining and not to cytoplasmic P-glycoprotein. Isotype control antibody does not react with either the MDR or the parental cell lines (Fig. 5).

Immunocytochemical detection of P-glycoprotein with 4E3 was further demonstrated in frozen sections of human tissues. Fig. 6A shows that 4E3 is able to detect P-glycoprotein along the luminal surfaces of bile canaliculi in frozen sections of human liver. In addition, 4E3 staining of human colon shows P-glycoprotein to be primarily localized to the luminal surface of the secretory epithelium (Fig. 6B). These patterns of expression are consistent with those previously described (8, 13–17) using other anti-P-glycoprotein antibodies and indicate the utility of 4E3 for the detection of mdr1 P-glycoprotein in tissue specimens.

4E3 Effects on Cytotoxicity. 4E3 alone had no effect on cell growth of either the drug-sensitive parent or MDR cell lines (data not shown). The ability of 4E3 to affect cell growth and survival in the MDR cell line CEM/VBL 300 was determined in the presence of cytotoxic agents. No significant potentiation of daunomycin cytotoxicity was observed at 4E3 concentrations of 10 pg/ml, 50 pg/ml, and 100 pg/ml (Fig. 7, A–C). In addition, no effect was observed with either vinblastine or actinomycin D when 4E3 was used at 10 pg/ml. A moderate potentiation of vinblastine (Fig. 7, E and F) and actinomycin D (Fig. 7, H and I) cytotoxicity on MDR cells was observed when 4E3 was used at 50 pg/ml and 100 pg/ml. This effect was not as great, however, as that observed with 5 μM cyclosporin A (Fig. 7). Similar results were obtained with the mdr1 cDNA transfectants and...
no potentiation of cytotoxicity was observed in drug-sensitive cell lines (data not shown).

Consistent with the results on the potentiation by 4E3 of cell growth inhibition by cytotoxic drugs is the inability of 4E3 to increase intracellular $[^{3}H]$daunomycin accumulation in MDR cells (Fig. 8A). The same holds true for $[^{3}H]$vinblastine when 4E3 was used at 10 µg/ml (Fig. 8B). However, there was a measurable increase in intracellular $[^{3}H]$vinblastine in MDR cells when 4E3 was used at 50 µg/ml and 100 µg/ml (Fig. 8B).

**DISCUSSION**

The Mab 4E3 is a mouse monoclonal antibody directed to an external epitope of the human mdrl-encoded P-glycoprotein. This Mab is effective at quantitatively detecting mdrl P-glycoprotein expression in flow cytometry on live cells. In addition, it is effective at detecting P-glycoprotein in immunohistochemistry on frozen sections of human tissues.

Immunoprecipitation of P-glycoprotein by 4E3 is most effective under mildly denaturating conditions. Complete denaturation results in the loss of binding of 4E3 to P-glycoprotein. Further confirmation of this point is demonstrated by the inability of 4E3 to detect P-glycoprotein by Western blot analysis following electrophoresis in SDS-containing polyacrylamide gels (data not shown). This is consistent with the manner in which 4E3 was generated, i.e., screening was against live cells expressing native P-glycoprotein. 4E3 recognizes both the nonglycosylated and glycosylated forms of P-glycoprotein. These data suggest that the 4E3 Mab recognizes a conformational peptide epitope of the P-glycoprotein. Functional analyses demonstrate that 4E3 does not increase the intracellular accumulation or the cytotoxicity of daunomycin in multidrug-resistant cells. Similarly, no effect on cytotoxicity of vinblastine and actinomycin D is observed up to 10 µg/ml 4E3. However, when 4E3 is present at 50 µg/ml or 100 µg/ml, a measurable increase of vinblastine and actinomycin D cytotoxicity is observed, although this increase is considerably less than that observed with classical MDR reversal agents such as cyclosporin A.

Several features of the 4E3 monoclonal antibody distinguish it from other monoclonal antibodies which have been previously described. MRK16, an IgG2a isotype antibody, was reported both to significantly increase intracellular accumulation and to potentiate the cytotoxicity of Vinca alkaloids and actinomycin D in MDR cells at antibody concentrations as low as 0.1 µg/ml (42). MRK17, an IgG1 isotype antibody, was reported to have no effect on drug accumulation or the potentiation of drug cytotoxicity but was found to inhibit the growth of MDR cells (42). The monoclonal antibody UIC2 binds to an external epitope of the P-glycoprotein and has been reported to efficiently potentiate the cytotoxicity of all P-glycoprotein-transported drugs to a greater extent than does the MDR reversal agent verapamil (43).

The monoclonal antibodies HYB-612, HYB-241, and HYB-195 have been reported to bind to an external epitope of a M, 180,000 membrane glycoprotein, but not to the M, 170,000 P-glycoprotein which is recognized by C219 (44). However, the immunoprecipitation with these antibodies was performed under relatively stringent denaturation conditions with both Triton X-100 and SDS. The results reported here using the 4E3 Mab, as well as data using MRK16 (42) and UIC2 (43), suggest that monoclonal antibodies to external regions of the P-glycoprotein are likely to be conformational epitopes sensitive to stringent denaturation conditions. Therefore, the inability to immunoprecipitate the M, 170,000 P-glycoprotein species reported for the HYB Mabs may be due to the detergent conditions utilized. This suggests that the M, 170,000 P-glycoprotein may be responsible for the functional effects observed with these antibodies. The HYB monoclonal antibodies were shown to increase intracellular Vinca alkaloid and actinomycin D levels in MDR cells at concentrations of antibodies as low as 1 µg/ml (44, 45). In contrast to these reports, 4E3 does not affect either drug accumulation or cytotoxicity in MDR cell lines when used at a saturating concentration of 10 µg/ml. This characteristic should be useful for isolating live cells expressing the P-glycoprotein and enables functional studies of P-glycoprotein-mediated substrate efflux to be performed without interference from the 4E3 Mab. In addition, 4E3 does not show any significant growth inhibition of MDR cells, thus allowing them to be grown in culture.

The 4E3 anti-P-glycoprotein monoclonal antibody should be particularly useful in the study of P-glycoprotein expression in both normal and neoplastic cells. In addition, it can be utilized to isolate live cells expressing P-glycoprotein from mixed cell suspensions for further functional analyses. Finally, the 4E3 Mab may prove useful therapeutically when conjugated to either biological toxins or cytotoxic drugs or may be useful for in vivo radiographic imaging of P-glycoprotein-expressing tumors (45, 46).
Further experimentation will be required to establish a role for such antibodies in the clinical setting.

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