Decreased P-Glycoprotein Expression in Multidrug-sensitive and -resistant Human Myeloma Cells Induced by the Cytokine Leukoregulin

Charles H. Evans and Patricia D. Baker

ABSTRACT

Modulation of the expression of P-glycoprotein, a plasma membrane protein associated with multidrug resistance, was examined in drug-sensitive and drug-resistant tumor cells treated with leukoregulin, a Mr 50,000 cytokine from human lymphocytes that rapidly permeabilizes the plasma membrane of many tumor cells facilitating the uptake of doxorubicin and other tumor-inhibitory antibiotics. P-glycoprotein expression was measured flow cytometrically by the binding of C219 or MRK16 monoclonal antibody to multidrug-sensitive human K562 erythroleukemia and 8226/S myeloma cells, compared to multidrug-resistant 8226/DOX_{40} myeloma cells. Cells were treated for up to 2 h with up to 80 units of leukoregulin/ml or one of a variety of unrelated cytokines including interleukin 1α (IL-1α), IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, colony-stimulating factor, macrophage colony-stimulating factor, granulocyte macrophage colony-stimulating factor, tumor necrosis factor α, γ-interferon, α-interferon, epidermal growth factor, platelet-derived growth factor BB, insulin-like growth factor I, insulin-like growth factor II, fibroblast growth factor, or transforming growth factor β. Leukoregulin caused a concentration-dependent decrease in P-glycoprotein expression; however, P-glycoprotein expression was unaffected by the other cytokines (<12% decrease in expression). Leukoregulin-induced membrane permeabilization, determined flow cytometrically by intracellular fluorescein efflux, and decreased P-glycoprotein expression occurred simultaneously within 15 min in drug-sensitive and -resistant cells. Enhanced doxorubicin uptake, measured flow cytometrically by doxorubicin influx, was also present within 15 min. Leukoregulin enhancement of doxorubicin uptake and increased membrane permeability varied directly with the decrease in P-glycoprotein expression. Leukoregulin in combination with doxorubicin enhanced the inhibition of cell proliferation in 8226/DOX_{40} multidrug-resistant cells by expressing P-glycoprotein. In contrast, combined treatment of HL-60/MX2 multidrug-resistant human promyelocytic leukemia cells that do not overexpress P-glycoprotein in association with their multidrug resistance resulted in no greater growth inhibition than observed with HL-60/MX2 cells treated with doxorubicin alone. This is the first demonstration that a naturally occurring macromolecule with anticancer activities can modulate the expression of P-glycoprotein concomitant with enhanced drug uptake and inhibition of cell proliferation.

INTRODUCTION

Overexpression of P-glycoprotein (1–5), a Mr 170,000 plasma membrane glycoprotein (1, 6–9) encoded by the mdr1 gene (10, 11), is a major factor implicated in the multidrug-resistant phenotype (12, 13). Evidence suggests that P-glycoprotein acts as an energy-dependent drug efflux pump, resulting in decreased net drug accumulation in cells (14–19). Reversal of multidrug resistance in vitro and in vivo by calcium channel blockers such as verapamil or calmodulin inhibitors such as trifluoperazine has been reported by several investigators (20–27). Multidrug resistance has also been reversed in vitro by nontoxic lipophilic drugs (28, 29). Most agents reversing multidrug resistance appear to competitively bind to P-glycoprotein, thereby blocking drug efflux and resulting in increased drug accumulation and enhanced cellular cytotoxicity (17, 18, 30–33).

Leukoregulin is a Mr 50,000 cytokine isolated from stimulated normal human peripheral blood lymphocytes (34). Leukoregulin treatment of many different tumor cell types permeabilizes the plasma membrane, facilitating the entry of pharmacologically active molecules such as doxorubicin and resulting in enhanced cellular cytotoxicity (35). Dalton and associates (36) have characterized a doxorubicin-resistant human myeloma cell line derived from the well-established human myeloma cell line RPMI 8226 (37). This drug-resistant derivative cell line, selected for its resistance to doxorubicin, expresses the multidrug phenotype with cross-resistance to mitoxantrone, acracycin, etoposide, and vincristine. Immunoblot analysis of plasma cell membrane proteins with the C219 monoclonal antibody (38), specific for P-glycoprotein, demonstrates overexpression of P-glycoprotein in resistant 8226/DOX_{40} cells compared to the sensitive 8226/S parent cells (36). Leukoregulin concomitant with increasing plasma membrane permeability rapidly modulates the expression of plasma membrane leukocyte and histocompatibility antigens in tumor cells as well as viral E6 and E7 gene expression in human papilloma virus 16 DNA-infected cervical epithelial cells (39, 40). In this study we have used the C219 and MRK16 monoclonal antibodies respectively directed against intracellular and extracellular epitopes on P-glycoprotein to determine flow cytometrically if leukoregulin can simultaneously modulate the expression of P-glycoprotein and drug uptake in multidrug-sensitive and -resistant 8226 myeloma cells.

MATERIALS AND METHODS

Target Cells and Culture Conditions. K562 human erythroleukemia cells, of the subline originally used to define leukoregulin (34), were cultured in RPMI 1640 (Life Technologies/Gibco, Grand Island, NY) containing 1% ITS+ (Collaborative Research, Inc., Bedford, MA). Multiple drug-sensitive (8226/S) and drug-resistant (8226/DOX_{40}) human myeloma cells (36) were obtained from Dr. William S. Dalton (University of Arizona Cancer Center, Phoenix, AZ). The sensitive HL-60 promyelocytic leukemia cell line and a multidrug-resistant variant, HL-60/MX2 (41), were a gift from Dr. W. Graydon Harker (Department of Veterans Affairs Medical Center, Salt Lake City, UT). HL-60/MX2 cells were previously made multidrug-resistant by a culture of HL-60 cells with increasing concentrations of mitoxantrone and are cross-resistant to etoposide, teniposide, bisantrene, dactinomycin, 4-(4-acridinylamino)methanesulfon-m-anisidine, and the anthracyclines daunorubicin and doxorubicin but retain sensitivity to the Vinca alkaloids, melphalan and mitomycin C. Multidrug-resistant HL-60/MX2 cells do not express P-glycoprotein as analyzed by Western blot using the C219 monoclonal antibody. Transcriptional RNA is also not detectable by Northern blot hybridization analysis using the mdr1 gene.
probe of Ueda et al. (49).2 8226/S, HL-60, and HL-60/MX2 cells were cultured in RPMI 1640 containing 10% FBS.3 8226/Dox cells were cultured in RPMI 1640 containing 10% FBS and 0.5 μm doxorubicin (Sigma Chemical Co., St. Louis, MO) to maintain the multidrug-resistant phenotype (36). HL-60/MX2 cells retain their 35-fold resistance to mitoxantrone for at least 3 months when grown in the absence of drug. All cell lines were incubated at 37°C in a water-saturated 5% CO2/95% air atmosphere.

Cytokine Preparations. Leukoregulin was isolated from phytohemagglutinin-stimulated normal human peripheral blood lymphocytes by sequential pH 7.4 diethylaminoethyl-anion exchange, pH 4.9—5.2 ionophore A23187 (Sigma Chemical Co.), or phospholipase C (Calbiochem, San Diego, CA) as previously described (43). After incubation, the cells were washed twice in PBS-1% BSA, and 20 μl of reconstituted antibody was added. The cell-antibody mixture (5 × 106 cells and 1 μg antibody) was incubated at 4°C for 60 min. Next the cells were washed twice in PBS-1% BSA (4°C), resuspended to 250 μl with PBS-1% BSA, and kept on ice until analyzed flow cytometrically.

Cells were also labeled in the presence of the MRK16 monoclonal antibody, kindly provided by Dr. Takashi Tsuruo (Japanese Foundation for Cancer Research, Tokyo, Japan). MRK16 is a murine (IgG2a) monoclonal antibody specific for a surface epitope of P-glycoprotein (44). The unfixed cells (5 × 109) were washed, labeled with 5 μg/ml MRK16 or 5 μg/ml purified myeloma protein mouse IgG2a (Organon Teknika-Cappel, West Chester, PA) as a negative control in a 100-μl volume for 30 min at 4°C, washed and labeled with human adsorbed FITC-goat Fab antiboys IgM plus IgG1 diluted 1:10 (Tago, Inc., Burlingame, CA) for 30 min at 4°C, washed, resuspended in PBS containing 0.4% BSA, and kept on ice until analyzed flow cytometrically.

P-glycoprotein expression was measured flow cytometrically using a fluorescence-activated cell sorter analyzer (Becton Dickinson Immunocytometry Systems, Mountain View, CA) equipped with a 75-μm orifice, a 3-decade log amplifier, and a Consort 40 data management system. Cells were excited by a mercury arc lamp at 488 nm using a combination of a 400-nm long pass, a 485 ± 22 nm band pass, and a 505-nm short-pass filter (43). Fluorescein emission was collected by means of a 530 ± 30 nm band pass filter. Calibration was performed with 10-μm-diameter Fullbright GR 1 beads (Coulter Electronics, Inc., Hialeah, FL), and cell volume was used to trigger all list mode data acquisition. Logarithmic amplification was set at 0.35 Ma; the photomultiplier tube setting was standardized by placing glutaraldehyde-fixed chicken RBC in fluorescent channel 122 of the 256-channel amplification scale, which placed the signal from nonlabeled nonmodulated cells in the lower 10% of the fluorescence signal channel range. Analysis of list mode data was performed by means of the standard version 5.0 Becton Dickinson software. Data from 20,000 cells were collected for each sample. Data were gated on cell volume to ensure analysis of single cells, excluding any small percentage of doublet or aggregated cells. To measure the decreased expression of P-glycoprotein a baseline window or region of interest was defined at the lower and upper boundaries of the higher fluorescence emission channels enclosing cells treated with medium and stained with either the FITC C219 or MRK16 antibody. The percentage decrease in P-glycoprotein expression was calculated by comparing the number of cells within the fluorescence emission window in the medium-treated population with the number of cells in the cytokine-treated population remaining in the baseline window:

% decreased P-glycoprotein expression = 100 — ( % cytokine treated population × 100)

Western Blot Analysis of P-Glycoprotein Expression. The method described by Tanaka et al. was used (45). Briefly, 106 cells were incubated for 2 h with 0—16 units of leuokergolin/ml and washed, and 107 8226/S or 108 8226/Dox cells were suspended in 100 μl 10 mM Tris-HCl (pH 8.0), 0.1% Triton X-100, 10 mM MgSO4, 2 mM CaCl2, 10 μg/ml DNase, 1 mM dithiothreitol; freeze-thawed three times; sonicated; incubated at 37°C for 10 min; and centrifuged at 12,000 × g for 10 min, and the supernatant containing P-glycoprotein was removed. SDS was added to this supernatant to a final concentration of 1%, glyceral to a final concentration of 10%, and bromophenol blue to a

2 W. G. Harker, personal communication.
3 The abbreviations used are: FBS, fetal bovine serum; IFN, interferon; IFN-γ, ILGF, platelet-derived growth factor AA and BB; MCSF, macrophage colony-stimulating factor; GMCSF, granulocyte macrophage colony-stimulating factor; EGF, epidermal growth factor; BSA, bovine serum albumin; IL, interleukin; TGF-β, transforming growth factor β; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; SDS, sodium dodecyl sulfate; SSC, standard saline citrate; TNF-α, tumor necrosis factor α; LR, leukoregulin.

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final concentration of 0.01%. Marker proteins for molecular weight calibration of the gel were obtained from Bio-Rad Laboratories (Richmond, CA). Myosin was used as the marker for Mr 205,000 and β-galactosidase was used for Mr 116,500. Proteins were separated on a 10.2 × 8.3 × 1.5 mm thick SDS-7% polyacrylamide gel at 40 mA constant current for 90 min according to the method of Laemmli (46) and electrophoretically transferred to a nitrocellulose membrane using Towbin’s procedure (47). The membrane was probed with 5 μg/ml C219 monoclonal antibody followed by goat anti-mouse horseradish peroxidase conjugate (Bio-Rad Laboratories) and developed using diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) containing 0.3% H2O2.

RNA Blot Hybridization. 8226/DOX40 cells (2 × 10⁶) were treated in a total volume of 1 ml with 0, 4, 8, or 16 units LR/ml for 2 h at 37°C in 5% CO2, divided into 500-μl aliquots, pelleted, washed once with PBS, and flash frozen in liquid N2. The pellets were stored at −70°C until used. Untreated 8226/S cells (1 × 10⁶) were used as a control.

Total RNA was extracted from 1 × 10⁶ cells by the acid-phenol method described by Chomczynski and Sacchi (48). The yield of total RNA was determined by measuring the absorption of each sample at 260 nm. For each sample, 3 and 1 μg of total RNA were slot blotted onto a Hybond-N nylon membrane (Amersham Corp., Arlington Heights, IL) using a Schleicher and Schuell (Keene, NH) slot blot apparatus. The air-dried membrane was fixed by irradiating it in a Strata-linker (Stratagene, La Jolla, CA) with 120,000 μJ of UV radiation. The membrane was then baked for 2 h at 80°C without vacuum.

Twenty-five ng of the EcoRI fragment of pHAMDR (49) and the SalI fragment of pl4b29Bl (50) were labeled by random priming using the Strata-linker (Stratagene, La Jolla, CA) with 120,000 μJ of UV radiation. The membrane was prehybridized for 30 min at 65°C with 3 ml prewarmed Quikhyb (Stratagene) in a Beilco (Vineland, NJ) autoblot apparatus. The air-dried membrane was fixed by irradiating it in a Strata-linker (Stratagene, La Jolla, CA) with 120,000 μJ of UV radiation. The membrane was then baked for 2 h at 80°C without vacuum.

The membrane was prehybridized for 30 min at 65°C with 3 ml prewarmed Quikhyb (Stratagene) in a Bellco (Vineland, NJ) autoblot microhybridization oven. 1 × 10⁷ cpm were added to 300 μl of sheared salmon sperm DNA (10 mg/ml) and boiled for 2 min. The denatured probe was added to 3 ml of 65°C Quikhyb, mixed, then added to the prehybridized membrane, and the incubation was continued for 3 more hours at 65°C. The hybridized blot was washed twice at room temperature for 1 min in 2x SSC (1x SSC = 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.4) and 0.1% SDS followed by two washes at 65°C for 15 min and a final two washes in 0.1x SSC and 0.1% SDS at 65°C for 20–30 min. The washed membrane was blotted dry, wrapped in plastic wrap, and exposed to preflashed Amersham Hyperfilm (Amersham Corp.) for 3 days. Quantitation of the amount of transcripts present was done by counting the membrane for 12 h using an Ambis Quantprobe version 3.0 software (AMGIS, Inc., San Diego, CA).

Before rehybridization, the membrane was stripped by pouring 200 ml of boiling 0.1x SSC and 0.1% SDS over the filter. The membrane was incubated in this boiling solution for 15 min, and the wash was repeated three times. The stripped membrane was blotted dry, wrapped in plastic wrap, and exposed overnight to preflashed Amersham Hyperfilm as described above.

RESULTS

The ability of leukoregulin to rapidly permeabilize the plasma membrane of tumor cells facilitating the entry of the tumor-inhibitory antibiotic doxorubicin results in enhanced cytotoxicity for K562 leukemia, 8226/S myeloma, and the P-glycoprotein-overexpressing 8226/DOX40 myeloma cells as shown in Fig. 1. Leukoregulin also affects HL-60 promyelocytic leukemia cells, but neither alone nor in combination with doxorubicin is it cytotoxic for the multiple drug-resistant HL-60/MX2 cells which do not overexpress P-glycoprotein. Drug-sensitive K562 and 8226/S cells as well as drug-resistant 8226/DOX40 cells constitutively express P-glycoprotein when stained with the FITC-C219 antibody, which binds to an intracellular epitope of P-glycoprotein, with the fluorescent intensity of multidrug-resistant 8226/DOX40 cells being approximately 110 arbitrary fluorescent units, 20 fluorescence units greater than that of the drug-sensitive cell lines. When the MRK16 monoclonal antibody is used, no detectable expression of a P-glycoprotein extracellular epitope is found on the 8226/S cells compared to the 8226/DOX40 P-glycoprotein-overexpressing myeloma cells, consistent with the overexpression of P-glycoprotein in the 8226/DOX40 cells (Fig. 2).

Leukoregulin diminishes the expression of P-glycoprotein in a dose-dependent manner in each cell line as shown by the decrease in fluorescence peak heights in the 90–110 fluorescence unit range. Leukoregulin (0.8 units/ml) diminishes P-glycoprotein expression in 6% of the K562, 5% of the 8226/S, and 8% of the 8226/DOX40 cells, while 8 units/ml reduce expression in 33%, 26%, and 30% of the cells, respectively. While the percentage decreases for the sensitive and resistant cells are similar, the absolute decrease of P-glycoprotein expression in the 8226/DOX40 cells following leukoregulin treatment is much greater due to the higher level of P-glycoprotein expression in the multidrug-resistant cells. Moreover, the diminution of P-glycoprotein expression is not gradual in relation to leukoregulin concentration but is largely complete, as evidenced by the shift in the fluorescence of the FITC-C219 labeled P-glycoprotein from the window region to the much lower levels of fluorescence exhibited by cells binding the nonfluorescent C219 antibody (Fig. 2). A similar decrease in the expression of P-glycoprotein resulting from leukoregulin treatment is shown in Fig. 2 for 8226/DOX40 cells incubated with either the C219 or MRK16 monoclonal antibody. For 8226/DOX40 cells exposed to 8 units leukoregulin/ml for 2 h, 30% of the cells exhibited decreased reactivity with the C219 antibody, as shown by the decrease in fluorescence from the region of interest compared to 35% with the MRK16 antibody, demonstrating a similar...
The decreased expression of P-glycoprotein is highly specific for leukoregulin when the decrease is compared to the modulation obtained with other cytokines (Fig. 4) or the membrane-permeabilizing agents calcium ionophore A23187 or phospholipase C (Fig. 5). IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, CSF, MCSF, GMCSF, TNF-α, IFN-α, IFN-γ, PDGF-AA, PDGF-BB, IGF-1, IGF-II, EGF, fibroblast growth factor, or TGF-β, over a 3-log concentration range, fail to modulate P-glycoprotein expression, as shown by the lack of change in the fluorescent intensity of P-glycoprotein expression in greater than 88% of the cells in all three cell lines. In contrast, in leukoregulin, phothyomagglutinin, calcium ionophore A23187, and phospholipase C, which increase plasma membrane permeability by a different mechanism (43, 52), affect P-glycoprotein expression in different ways. Calcium ionophore A23187 and phospholipase C do not alter the level of P-glycoprotein expression in either 8226/S or 8226/DOX40 cells, while phothyomagglutinin markedly decreases P-glycoprotein expression in 8226/S cells to a degree greater than that induced by leukoregulin (Fig. 5). Unlike leukoregulin, however, no concentration-dependent decrease in P-glycoprotein expression is obtained following phothyomagglutinin treatment. P-glycoprotein expression in 8226/DOX40 cells treated with either 0.5 or 6.4 μM phothyomagglutinin, a 13-fold increase in phothyomagglutinin concentration, is decreased to approximately the same level as cells treated with leukoregulin.

Leukoregulin induced decreased P-glycoprotein expression, increased membrane permeability, and enhanced doxorubicin uptake simultaneously but not to the same degree (Fig. 6). Within 15 min, membrane permeabilization is increased, P-glycoprotein expression is decreased, and doxorubicin uptake is decreased in P-glycoprotein expression as measured by antibodies to two different epitopes on the P-glycoprotein. Western blot analysis of proteins extracted from the cell membranes using the C219 antibody also demonstrates decreased P-glycoprotein in 8226/DOX40 cells after leukoregulin treatment (Fig. 3).

RNA from leukoregulin-treated and untreated cells was examined by RNA slot blot hybridization to determine if the decrease in P-glycoprotein expression correlates with a decrease in steady-state levels of transcripts of the mdrl multidrug resistance gene. No diminution in the steady-state levels of multidrug resistance transcriptional RNA was observed. The maximum changes observed were a less than 2-fold increase in mdrl steady-state transcriptional RNA in 8226/DOX40 cells treated with 8 or 16 units of leukoregulin/ml when the level of multidrug-resistant transcript was measured relative to a normalized constant level of β-actin gene RNA expression for each of the leukoregulin treatments. No multidrug-resistant transcripts were found in the multidrug-sensitive 8226/S myeloma cells.

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enhanced in leukoregulin-treated K562 cells. Multidrug-sensitive 8226/S cells and resistant 8226/DOX or cells exhibit maximum membrane permeabilization at 60 min, with 68% of the sensitive and 67% of the resistant cells demonstrating increased permeability accompanied by diminished P-glycoprotein expression in 25% of the sensitive and 24% of the resistant cells (Fig. 6). In both multidrug-sensitive and -resistant cells the extent of P-glycoprotein diminution is less and reaches the maximum decrease more quickly than the increased plasma membrane permeability induced by leukoregulin.

The leukoregulin-induced decrease of P-glycoprotein expression in both multidrug-sensitive and -resistant cells varies directly with the increase in plasma membrane permeability and enhanced uptake of doxorubicin as demonstrated in Fig. 7. Drug-sensitive 8226/S or resistant 8226/DOX or cells treated with 0.5 μM doxorubicin or doxorubicin combined with 8 units leukoregulin/ml for 60 min show a 2-fold enhancement of drug uptake with the doxorubicin-leukoregulin combination treatment (P < 0.05 for sensitive 8226/S cells and P < 0.0001 for resistant 8226/DOX or cells) as shown in Fig. 7. Eighty-eight % of untreated 8226/S cells express P-glycoprotein, and after treatment with 8 units leukoregulin/ml for 60 min P-glycoprotein expression is expressed by 62% of the cells, a 30% decrease in P-glycoprotein expression (P < 0.0001). Eighty-eight % of the multidrug-resistant 8226/DOX or cells express P-glycoprotein, and after leukoregulin treatment P-glycoprotein is expressed by 78% of the cells, an 11% decrease in P-glycoprotein expression.
decrease per affected cell is greater for the multidrug-resistant expression in the affected cells (Fig. 2) and that the absolute decrease is indicative of a complete loss of P-glycoprotein (P < 0.0001). It should be noted again that the percentage decrease in plasma membrane permeability within 5—15 mm in cells exposed to leukoregulin (43) is accompanied by an increase in calcium metabolism (52), protein kinase C activation and translation from the cytosol to the plasma membrane (53), plasma membrane changes in fluidity and conformation (54), and changes in plasma membrane antigen expression (39) with accompanying alterations in gene transcription and translation (40). Associated with the leukoregulin-induced increase in plasma membrane permeability is a change in membrane conformation detected by fluorescence depolarization of cell surface-bound fluorochrome-conjugated concanavalin A and a change in membrane fluidity detected by the fluorescence depolarization of membrane-localized 1,6-diphenyloxatrene (54). Also accompanying these leukoregulin-directed plasma membrane changes are the activation of transmembrane cation channels (55) and the modulation of leukocyte (39) and histocompatibility (40) cell membrane antigens. These biochemical and physiological alterations indicate the powerful ability of leukoregulin to alter the quantity as well as the tertiary and quaternary structures of membrane proteins, which can singly and collectively alter transmembrane signals, molecular transport, and other homeostatic functions.

Modulation of P-glycoprotein synthesis (1—6), phosphorylation (56), and conformation (reviewed in Ref. 57) and the ability of nontoxic lipophilic drugs to bind to P-glycoprotein (29) have been proposed as mechanisms responsible for controlling the multidrug-resistant phenotype. The decrease in anti-P-glycoprotein monoclonal antibody binding measured in situ flow cytometrically or by Western blot analysis of extracted proteins from leukoregulin-treated multidrug-resistant 8226/DOX40 myeloma cells in the present investigation suggests that the leukoregulin modulation of P-glycoprotein expression results from a decrease in synthesis or translocation of P-glycoprotein from the cell membrane. The decrease in extractable P-glycoprotein shown by the Western blot analysis does not support the alternative possibility that the modulation of P-glycoprotein expression is the result of the binding of leukoregulin itself to the P-glycoprotein. Whereas the Western blot analysis weighs in favor of a decreased synthesis or transport of P-glycoprotein to the membrane, future investigations are required to rule out a primary or secondary induced permanent conformational change in P-glycoprotein altering both intracellular and extracellular epitopes resulting from leukoregulin treatment of the cells. The lack of a demonstrable decrease in mdrl gene transcripts as detected by the RNA slot blot hybridization analysis provides further support for the possibility of leukoregulin-induced posttranscriptional alteration of P-glycoprotein expression. Delineation of these possibilities is an important avenue of future investigations, both in terms of defining the molecular pathways of leukoregulin action and elucidation of the pathways critical to expression and function of the multidrug-resistant phenotype.

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