Expression and Activity of the Multidrug Resistance P-Glycoprotein in Human Peripheral Blood Lymphocytes

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P-glycoprotein (P-gp), the product of the MDR1 (multidrug resistance) gene, is a transmembrane efflux pump for different lipophilic compounds, including many anticancer drugs and fluorescent dyes. We have previously reported that the efflux of fluorescent dyes from lymphoid cells of human bone marrow was directly correlated with the cellular P-gp content. In the present study, we show that human peripheral blood lymphocytes (PBL) also express P-gp, and that P-gp expression correlates with the efflux of fluorescent dyes from PBL. This efflux was suppressed not only by chemical inhibitors of P-gp but also by a P-gp-specific monoclonal antibody UIC2, thus providing direct evidence that it was mediated by P-gp. We have also characterized dye efflux and UIC2 reactivity in specific PBL subsets. P-gp was expressed in the majority of CD56+, CD8+, and CD20+ lymphocytes, but in less than one half of CD4+ cells. P-gp-mediated dye efflux was highly heterogeneous relative to the expression of CD56RA, CD66RO, Leu-8, and HLA-DR antigens. No significant P-gp activity was detectable in CD14+ monocytes. MDR1 expression in normal lymphocytes may be a determinant of multidrug resistance in the corresponding malignancies.

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MATERIALS AND METHODS

Antibodies and fluorescent dyes. Monoclonal antibody (MoAb) UIC2 (IgG2a) against an extractoplasmonic domain of P-gp has been previously described.13 MoAb UIC2 was purified from the mouse ascitic fluid using a protein A column, followed by extensive dialysis against phosphate buffer saline or serum-free Dulbecco’s modified Eagle’s medium. A nonspecific isotype control MoAb UCPI0 (affinity purified IgG2a myeloma protein) was purchased from Sigma (St Louis, MO). All MoAbs were at least 95% pure, as judged by sodium dodecyl sulfate (SDS) electrophoresis.

P-glycoprotein (P-gp) is a transmembrane efflux pump for a diverse group of lipophilic compounds, including many anticancer drugs such as anthracyclines, vinca alkaloids, and flavonoids. The expression of P-gp has been associated with clinical resistance to chemotherapy, indicating that P-gp-mediated multidrug resistance is an important clinical problem.5 The knowledge of the expression and regulation of the MDR1 gene in normal tissues is essential for understanding the origin of multidrug resistance in clinical cancer.

Initial studies on MDR1 expression in normal human tissues have identified the adrenal cortex, luminal surfaces of the kidney, liver, jejunum and pancreas, placental trophoblasts, and endothelial cells in the brain and testes as the major P-gp-expressing sites.9,10 It was only recently that the expression of the MDR1 gene was found in normal cells of the peripheral blood and bone marrow. The initial evidence came from the study by Neyfakh et al11 who showed that some of the normal mouse and human lymphocytes rapidly effluxed Rh123 and that such efflux could be blocked by known chemical inhibitors of P-gp. We have recently shown that the efflux of Rh123 and several other fluorescent dyes from normal human bone marrow cells was not only sensitive to P-gp inhibitors but also directly correlated with the expression of P-gp on the cell surface.12 P-gp expression and activity were associated with all early hematopoietic progenitors, as characterized by CD34 expression, clonogenic assays, and long-term culture initiating assays. P-gp expression in bone marrow samples was not confined solely to CD34+ cells but was found in greater than 50% of all lymphoid marrow cells.12

In the present study, we have shown directly that the efflux of fluorescent dyes from human peripheral blood lymphocytes (PBL) is determined by the P-gp function. We have also used functional and immunofluorescence assays for P-gp to show differential P-gp expression in specific PBL subsets. Our results indicate a specific pattern of P-gp expression in different subsets of PBL, suggesting that P-gp plays a physiological role in normal lymphocytes. Our results also suggest that MDR1 expression in untreated hematopoietic malignancies may reflect the expression of this gene in the normal cells from which such malignancies arise.
hydrated for 30 minutes, and washed before further manipulation. No major difference was detected in the dye efflux of the cells from fresh or frozen samples.

To study the dye efflux from peripheral blood mononuclear cells, the cells were loaded with 60 ng/mL DiOC$_3$(3) or with 150 ng/mL of Rh123 for 25 minutes in 10 mL of media at 4°C and, after two washes with ice-cold media, allowed to efflux the dye in 10 mL of dye-free media (RPMI with 10% fetal calf serum [FCS]) for 3 hours at 37°C or at 4°C. In parallel experiments, dye efflux was performed at 37°C in the presence of P-gp inhibitors: 30 μmol/L verapamil, 20 μmol/L reserpine, 20 μmol/L cyclosporin A or 2 μmol/L SDZ-33-243 (Sandoz, East Hanover, NJ). After efflux, cells were washed, stained with propidium iodide, and kept on ice until analysis. Flow cytometric analysis was conducted on a Coulter Epics 753 flow cytometer (Coulter, Hialeah, FL) as previously described.

For experiments involving inhibition of dye efflux by UIC2 MoAb, 10$^6$ peripheral blood lymphocytes from a normal donor were incubated with MoAb UIC2 or isotype control (UPC 10) at the concentration 20 μg/mL (shown to be saturating in preliminary experiments) for 30 minutes at 4°C, washed twice, and stained with Rh123 at 5 μg/mL for 15 minutes at 4°C. The cells were then washed twice with ice-cold incubation medium and allowed to efflux the dye in the presence of the MoAb (20 μg/mL) at 37°C for 5 hours. Aliquots of cells were taken at different periods of time, stained with propidium iodide and kept on ice until analysis. Cell fluorescence was analyzed in lymphoid and monocyte bit maps.

**RESULTS**

Efflux of fluorescent dyes from human peripheral blood lymphocytes is determined by P-gp. We have analyzed the role of P-gp in the efflux of fluorescent mitochondrial dyes, DiOC$_3$(3), and Rh123, from human peripheral blood lymphocytes. DiOC$_3$(3) is effluxed more rapidly from P-gp-expressing cells and has more favorable fluorescence spectra for multicolor flow cytometry than Rh123.
To determine if the efflux of fluorescent dyes from lymphocytes correlated with their P-gp expression, the cells were stained with DiOC<sub>3</sub>(3) or Rh123 and, after efflux at 37°C, labeled by indirect immunofluorescence with MoAb UIC2 recognizing an extracellular epitope of the human MDR1-encoded P-gp. P-gp expression was detectable in 40% to 65% of the PBL, depending on the individual sample. Furthermore, there was a strong inverse correlation between the levels of P-gp expression and the retention of fluorescent dyes in the PBL (Fig 2), indicating that the dye efflux was directly correlated with P-gp expression.

To show directly that the efflux of fluorescent dyes from PBL was due to the function of P-gp on the cell surface, we have taken advantage of the ability of UIC2 MoAb to inhibit specifically the efflux of P-gp substrates. As shown in Fig 3, there was pronounced inhibition of Rh123 efflux in the PBL samples treated with a saturating concentration (20 μg/mL) of UIC2 relative to samples treated with the same concentration of UPC10 (an unrelated antibody of the same IgG2a isotype). Preincubation of the cells with UIC2 had no effect on the initial staining with Rh123 at 4°C (data not shown). Inhibition of Rh123 efflux from PBL by UIC2 provides the direct evidence that this efflux is mediated by P-gp.

P-gp expression in specific subsets of PBL. To characterize P-gp expression in specific lymphocyte subtypes, we have studied the ability of various subsets of PBL to efflux fluorescent dyes transported by P-gp. PBL were stained with DiOC<sub>3</sub>(3) for 10 minutes and, after efflux at 37°C for 3 hours, reacted with MoAbs against CD8, CD4, CD56, CD20, CD45RO, CD45RA, Leu-8, or HLA-DR antigens. The proportion of DiOC<sub>3</sub>(3)-dull cells was determined for each antigenic subtype (Fig 4). Rh123 efflux assays (data not shown) produced essentially the same results as DiOC<sub>3</sub>(3) staining. The presence of P-gp in the corresponding subsets was confirmed by double immunofluorescence labeling with the subset-specific MoAb and the MoAb UIC2 against P-gp (Fig 5).

The majority (80% to 90%) of the CD8<sup>+</sup> (cytotoxic/suppressor) cells effluxed DiOC<sub>3</sub>(3). A similar proportion (70% to 80%) of CD8<sup>+</sup> cells also stained positively with UIC2. An even higher percentage of P-gp-positive cells was found in the CD56<sup>+</sup> (natural killer) cell population; 90% to 95% of these cells effluxed DiOC<sub>3</sub>(3) and stained with UIC2. In contrast, the population of dye-effluxing cells among the CD4<sup>+</sup> (helper/inducer) cells was only 40% to 50%. Similarly, 30% to 40% of CD4<sup>+</sup> lymphocytes stained positively with MoAb UIC2. The relative levels of UIC2 reactivity of positive cells were also different for...
different subtypes, in the order CD56+ > CD8+ > CD4+ (Fig 5).

DiOC₂(3) staining of CD20+ B lymphocytes showed that 60% to 80% of these cells effluxed the dye to various extent. However, P-gp expression on CD20+ cells could not be measured by indirect immunofluorescence labeling with UIC2, because of a high degree of nonspecific binding of the secondary antibody to the B cells. DiOC₂(3) efflux was also highly heterogeneous with regard to the expression of the following antigens: CD45R0, CD45RA, Leu-8, and HLA-DR (Fig 4).

We have also studied DiOC₂(3) efflux in CD14+ monocytes (Fig 6). In the absence of P-gp inhibitor, the majority...
of cells showed a uniform strong retention of the dye, except for a small subpopulation of cells that stained less brightly. However, no increase in DiOC2(3) staining of this subpopulation was observed in the presence of 50 μmol/L verapamil, indicating that its decreased staining was not associated with P-gp-mediated efflux. Furthermore, no specific inhibition of Rh123 efflux by MoAb UIC2 was observed in the monocyte bit map (data not shown). These results indicate the lack of functionally active P-gp in CD14+ monocytes.

**DISCUSSION**

In the present study we have documented that P-gp is expressed and active in a major subpopulation of human peripheral blood lymphocytes. Our conclusion, which confirms the earlier hypothesis of Neyfakh et al., is based on the following observations. Using DiOC2(3) and Rh123, two known fluorescent substrates of P-gp, we have shown that between 50% and 70% of normal peripheral blood lymphocytes effluxed the dye. This efflux was abolished if the cells were incubated at 4°C or in the presence of known nonspecific chemical inhibitors of P-gp. Furthermore, there was a direct correlation between dye efflux and expression of cell-surface P-gp. Finally, dye efflux from PBL was suppressed by MoAb UIC2, a highly specific P-gp inhibitor.

There was considerable heterogeneity in the percentage of PBL from different individuals that effluxed P-gp—transported fluorescent dyes or stained with anti-P-gp antibody (40% to 65%). There was also individual variability with regard to P-gp expression in specific antigenic classes. However, qualitatively, the overall pattern of P-gp expression in specific lymphocyte subsets was similar in different individuals. The highest proportion of P-gp-positive cells was found in the natural killer (CD56+) cells and killer.suppressor (CD8+) cells. Based on the dye efflux criterion, the majority of CD20+ B cells also appear to express P-gp. Among CD4+ helper/inducer cells, P-gp expressers constituted a little less than one half. P-gp distribution relative to the other tested lymphoid markers was highly heterogeneous. In contrast to PBL, no P-gp-mediated dye efflux was detectable in CD14+ monocytes.

We have previously shown that P-gp is expressed at relatively high levels in early hematopoietic progenitors, where its expression correlates with that of CD34 antigen. In the present study, we have shown that different lymphocyte subsets have different percentages of P-gp—expressing cells. The observed distribution of P-gp is in good agreement with the previous reports on Rh123 staining of several specific subsets of mouse and human PBL. Furthermore, our results show an excellent correlation with a study of Drach et al. who have analyzed the expression of MDR1 mRNA in individual subsets of peripheral blood and mononuclear cells by a cDNA-polymerase chain reaction (PCR) assay. This correlation suggests that P-gp expression in hematopoietic cells is regulated, at least in part, at the RNA level. The specific patterns of P-gp expression in hematopoietic cells suggest a physiologic role for P-gp in this tissue. Our results also suggest that expression of the MDR1 gene in normal lymphocytes may be a determinant of multidrug resistance in untreated hematopoietic malignancies arising from such cells.

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**REFERENCES**

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