P-glycoprotein (P-gp) is an ATP-dependent drug pump that confers multidrug resistance (MDR). In addition to its ability to efflux toxins, P-gp can also inhibit apoptosis induced by a wide array of cell death stimuli that rely on activation of intracellular caspases for full function. We therefore hypothesized that P-gp may have additional functions in addition to its role in effluxing xenotoxins that could provide protection to tumor cells against a host response. There have been a number of contradictory reports concerning the role of P-gp in regulating complement activation. Given the disparate results obtained by different laboratories and our published results demonstrating that P-gp does not affect cell death induced by another membrane-lytic protein, perforin, we decided to assess the role of P-gp in regulating cell lysis induced by a number of different pore-forming proteins. Testing a variety of different P-gp-expressing MDR cell lines produced following exposure of cells to chemotherapeutic agents or by retroviral gene transduction in the complete absence of any drug selection, we found no difference in sensitivity of P-gp<sup>+</sup>ve or P-gp<sup>−/−</sup>ve cells to the pore-forming proteins complement, perforin, or pneumolysin. Based on these results, we conclude that P-gp does not affect cell lysis induced by pore-forming proteins.

P-glycoprotein (P-gp),<sup>1</sup> a member of the ATP-binding cassette (ABC) superfamily, is encoded by the MDR1 gene in humans and mdr1a and mdr1b in mice and has been demonstrated to act as a very efficient toxin efflux molecule (1, 2). In the clinical setting, expression of P-gp on tumor cells confers resistance to a wide range of different chemotherapeutic agents constituting a multidrug-resistant (MDR) phenotype and a poor prognosis. The current working model maintains that P-gp removes xenotoxins in an energy (ATP)-dependent manner by intercepting the drug as it moves through the lipid membrane and flips the drug from the inner leaflet to the outer leaflet and into the extracellular media (3). Consistent with its toxic clearance role, P-gp is expressed on the surface of normal human cells found in the gut, liver, and kidney tubules, and at blood-tissue barriers (4). However, P-gp is also expressed in the adrenal gland, hemopoietic stem cells, natural killer (NK) cells, antigen-presenting dendritic cells, and T and B lymphocytes (5, 6), and a role for P-gp in removing xenotoxins from these cells is not immediately apparent.

Recent studies by our group and others have indicated that, in addition to its role as an efflux pump, P-gp also regulates programmed cell death mediated by some chemotherapeutic drugs, serum starvation, ultraviolet (UV) irradiation, and ligation of the cell surface death receptors Fas and tumor necrosis factor receptor (7–9). The ability of P-gp to inhibit cell death mediated by these diverse apoptotic stimuli appears to be due to P-gp-mediated inhibition of caspase activation. In contrast, other stimuli such as the chemotherapeutic agent hexamethylamine bisacetaetade (10), the protein kinase C inhibitor staurosporine (11), the CTL granule protein granzyme B (9) and the CTL pore-forming protein perforin (9), which are fully functional in the absence of caspase activation, were not affected by P-gp.

A number of groups have analyzed the potential effects of P-gp on cell lysis mediated by complement, a family of related pore-forming proteins necessary for antibody-mediated cytolyis. Initial studies focused on successfully using anti-P-gp antibodies and rabbit complement to purge autologous bone marrow grafts of residual P-gp<sup>−/−</sup> MDR cells (12), or complement fixing anti-P-gp antibodies to eliminate P-gp<sup>−/−</sup>ve tumors in mouse model systems (13). In agreement with these studies were those of Bomstein and Fishelson (14), who demonstrated that P-gp<sup>−/−</sup>ve tumor cell lines were more sensitive to complement-mediated lysis compared with matched P-gp<sup>−/−</sup>ve parental cells. However, in contrast to these findings were two reports demonstrating a role for functional P-gp in inhibiting membrane damage by complement (15, 16). The authors of these studies presented evidence suggesting that P-gp-mediated intracellular alkalization (pH<sub>i</sub>) and/or decreased plasma membrane potential (V<sub>max</sub>) resulted in a reduction in the rate of formation of the “membrane attack complex” (MAC) at the cell surface. Functional MACs consist of complement components C5b, C6, C7, C8, and multiple C9 molecules to form pores.
Expression of functional P-gp was shown to correlate with a reduction in the rate of MAC formation rather than the molecular stoichiometry of MAC complexes (16). It was hypothesized that altered pH$_i$ and/or $V_{max}$ may somehow directly or indirectly affect C9 polymerization, resulting in a net loss of functional MAC formation (16).

Perforin is essential for death of virus-infected or malignant target cells by CTL and NK effector cells (18). Perforin, expressed in the granules of CTL and NK cells mediates the transport of granzymes into the target cell to induce programmed cell death via a process known as granule exocytosis (18). Following the initial cloning and characterization of perforin, it was demonstrated that there were structural and functional similarities between perforin and the C9 component of complement (19, 20). Both could form pores in lipid membranes, both had conserved amino acid motifs, and monoclonal antibodies raised against purified C9 and perforin showed cross-reactivity (19, 20). We have previously demonstrated equivalent lysis of P-gp$^+$ and P-gp$^-$ CEM cells treated with perforin (9). In view of the structural and functional similarities between C9 and perforin and the contradictory findings by a number of groups regarding regulation of complement-mediated lysis by P-gp, we performed a comprehensive analysis of the role of P-gp in regulating pore formation. Using a number of matched P-gp$^-$ and P-gp$^+$ cell lines isolated following treatment of cells with chemotherapeutic agents, and different pore-forming proteins such as activated complement, purified recombinant perforin, and the bacterial toxin pneumolysin, we observed no effect of P-gp on cytolysis induced by these agents. These studies were confirmed using P-gp$^+$ multidrug-resistant cells produced by retroviral gene transduction and cloned by fluorescence-activated cell sorting in the complete absence of drug selection. Our data therefore indicate that P-gp does not affect the cytolytic effects of pore-forming proteins.

**MATERIALS AND METHODS**

**Cell Culture**—The acute T cell leukemia cell line, CEM-CCRF, its doxorubicin (DOX)-selected and -resistant P-gp$^+$ line CEM-A7$^+$, have been previously described (21). K562 and vincristine-selected P-gp$^+$ Kvin2000 cells were kindly provided by Greg Woods (University of Tasmania, Hobart, Australia). All cells were grown in RPMI medium 1640 supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 100 m$g$/ml streptomycin (Life Technologies, Inc.). The cell surface expression of P-gp and Fas was confirmed and monitored by fluorescence analysis using the MRK 16 anti-P-gp (Kamiya Biochemical Co., Thousand Oaks, CA) and CH-11 anti-human Fas (Upstate Biotechnology Inc., Lake Placid, NY) monoclonal antibodies, respectively. Drug efflux activities of P-gp and reversal with verapamil...
were assessed by rhodamine 123 exclusion assays as described (22).

Generation of MSCV-based Supernatant and Transduction of Mammalian Cell Lines—The MDR1 coding region was cloned into the retroviral vector plasmid MSCV. This bicistronic vector contains (i) the amphotropic retrovirus murine stem cell virus (MSCV) 5’-long terminal repeat, (ii) the encephalomyocarditis internal ribosomal entry site, (iii) the green fluorescent protein (GFP) cDNA, and (iv) the MSCV 3’-long terminal repeat (23). The plasmid was cotransfected with an amphotropic packaging plasmid into 293T cells by calcium phosphate precipitation. After 48 h, the supernatant containing amphotropic particles was harvested, filtered, and added to CEM-CCRF cells every 8 h for 3 days. The cells were allowed to recover for 72 h and then analyzed for GFP expression by flow cytometry. The highest expressing 10% of cells were sterilely sorted, expanded, resorted, and subsequently expanded in oligoclonal pools. Cells were subsequently screened for expression of P-gp and sorted by flow cytometry using the MRK-16 monoclonal antibody (Kamiya Biochemical Co.).

Membranolytic and Chemotherapeutic Agents—Human perforin was purified as described previously (24). Pneumococcal PLO was provided by Dr. James Paton (Childrens and Womens Hospital, Adelaide, Australia). Freshly prepared serum from naïve rabbits was used as the source of hemolytic complement. Small aliquots of serum were stored at −70 °C and discarded after each use. Heat inactivation at 65 °C for 30 min caused total inhibition of the complement activity. DOX and vinca-ristine were obtained from Dr. Phillip Kantharidis (Peter MacCallum Cancer Institute, East Melbourne, Australia).

Expression of Cell Surface CD35, CD46, CD55, and CD59—Expression of the regulators of complement activation, CD35, CD46, CD55, and CD59, on the cell surface was determined by flow cytometry. Monoclonal antibodies against CD35 (BD PharMingen, San Diego, CA), CD46 (E4.3, IgG2a), CD55 (clone 1H4, IgG1), and CD59 (clone MEM-43, IgG2a), kindly provided by Dr. S. Russell (Peter MacCallum Cancer Institute, Melbourne, Australia), or appropriate isotype control antibodies were incubated with P-gp−ve and P-gp+ve CEM cells on ice for 1 h. Cells were washed in phosphate-buffered saline containing 0.5% bovine serum albumin and incubated with fluorescein isothiocyanate anti-mouse immunoglobulin for 1 h on ice. Antibody binding was detected by flow cytometry using a FACScan (Beckton Dickinson).

Cytotoxicity Assays—Cell death was assessed by 51Cr release assays as described (8). The spontaneous release of 51Cr was determined by incubating the target cells with medium alone (or in the presence of anti-P-gp monoclonal antibody (mAb), verapamil, or caspase inhibitor where applicable). It should be noted, at the concentrations used, inhibitors alone did not cause release, nor did they affect the long term survival of cell lines. The maximum release was determined by adding SDS to a final concentration of 5%. The percentage of specific lysis was calculated as follows: 100 × ([experimental release − spontaneous release]/maximum release − spontaneous release). To inhibit P-gp function, the labeled targets were preincubated for 30 min with UIC2 (IgG2a mAb; final, 0.1–5 μg/ml) (Coulter, Miami, FL) or verapamil (0.5–10 μM) (Knoll Australia, Lane Cove, Australia) prior to the cytotoxicity assay. Isotype control antibodies were included where applicable. To inhibit caspase activity, labeled target cells were preincubated for an additional 30 min with peptidyl fluoromethyl ketones (ZFA- and

Fig. 3. Cell surface expression of complement regulatory proteins. K562 and Kvin cells were incubated with mAbs specific for CD35, CD46, CD55, and CD59 (filled histograms) or appropriate isotype controls (unfilled histograms), and fluorescein isothiocyanate-labeled anti-mouse secondary antibodies. Cell surface antibody binding was detected by flow cytometry.

Fig. 4. P-gp does not affect myeloid cell lysis induced by perforin or pneumolysin. 51Cr-Labeled K562 (P-gp−ve) and Kvin (P-gp+ve) cells were incubated with purified human perforin (Pfp, 1.0 hemolytic units/ml serially diluted 2-fold) for 4 h at 37 °C or vincristine (Vin, 100 ng/ml) 24 h at 37 °C (A) and PLO (1.44 μg/ml serially diluted 10-fold) for 4 h at 37 °C or doxorubicin (Dox, 100 ng/ml) for 24 h at 37 °C (B). Data are calculated as the mean ± S.E. of triplicate samples and are representative of at least two different experiments.

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FIG. 5. P-gp does not affect T lymphocyte cell lysis induced by complement, perforin, or pneumolysin. $^{51}$Cr-Labeled CEM (P-gp$^{+v}$) and A7 (P-gp$^{-v}$) cells were incubated with serially diluted (A) rabbit serum containing active complement (C9, 1:4–1:64) or heat-inactivated rabbit serum (HI C9, 1:4) for 1 h at 37 °C (A); purified human perforin (Pfp, 1.0 hemolytic units/ml serially diluted 2-fold) (B); or PLO (1.44 μg/ml serially diluted 10-fold) for 4 h at 37 °C (C). As a control for P-gp function, cells were treated with vincristine (Vin, 100 ng/ml) (A) or doxorubicin (Dox, 100 ng/ml) for 24 h at 37 °C (B and C). Data are calculated as the mean ± S.E. of triplicate samples and are representative of at least two different experiments.

FIG. 6. Production of P-gp-expressing CEM cells by retroviral gene transduction. CEM cells were transduced with the MSCV-MDR1 retrovirus, sorted for GFP expression by flow cytometry, and then resorted for expression of P-gp using the MRK 16 anti-P-gp monoclonal antibody. Cells expressing GFP and P-gp (RP-gp$^{+v}$) or GFP without P-gp (RP-gp$^{-v}$) were obtained. A, CEM, A7, RP-gp$^{+v}$, and RP-gp$^{-v}$ cells were analyzed for expression of cell surface P-gp (vertical axis) or intracellular GFP horizontal (horizontal axis). B, CEM, A7, RP-gp$^{+v}$, and RP-gp$^{-v}$ cells were assessed for P-gp function by $^{125}$I efflux assays. Fluorescence of resting (filled histograms) and $^{125}$I-treated (unfilled histograms) cells was analyzed by flow cytometry. An increase in fluorescence correlates with uptake of the fluorescent $^{125}$I dye.

FIG. 7. P-gp-expressing CEM cells produced by retroviral gene transduction are multidrug-resistant. $^{51}$Cr-Labeled CEM, A7, RP-gp$^{+v}$, and RP-gp$^{-v}$ cells were treated with various concentrations of vincristine (Vin) or doxorubicin (Dox) for 24 h at 37 °C. Data are calculated as the mean ± S.E. of triplicate samples and are representative of at least two different experiments.
RESULTS

P-gp Does Not Protect K562 Cells against Complement-mediated Lysis—A previous study had shown that P-gp^-ve K562 cells were less sensitive to complement-mediated lysis compared with parental P-gp^-ve K562 cells (15). In that study, complement deposition was induced by activating the classical complement pathway following addition of an anti-CD71 antibody (15). To test this finding, we used matched P-gp^-ve and P-gp^-ve K562 cells derived following incubation of the parental cell line in vincristine, and anti-CD71 or anti-FcγRII mAbs to activate the complement cascade. Equivalent binding of both antibodies to their target antigens on P-gp^-ve and P-gp^-ve K562 cells was demonstrated (Fig. 1). Addition of a single dose of complement to increasing concentrations of anti-CD71 (Fig. 2A, lanes 3–8) or anti-FcγRII (Fig. 2A, lanes 13–18) mAbs resulted in a dose-dependent increase in cell lysis. Cell death was dependent on activated complement, as heat inactivation of the rabbit serum completely inhibited ⁵¹Cr release (Fig. 2B, lanes 9–12, 19, and 20). The drug efflux function of P-gp expressed on the K562 cells was confirmed by addition of DOX to P-gp^-ve and P-gp^-ve cells. Only P-gp^-ve K562 cells were sensitive to DOX-induced cell death (Fig. 2A, lanes 21 and 22). To confirm these data and demonstrate that the dose of complement used in Fig. 2A did not overwhelm potential subtle differences in susceptibility of P-gp^-ve and P-gp^-ve K562 cells to complement-mediated lysis, a single dose of anti-FcγRII antibody was added to increasing concentrations of complement (Fig. 2B). Both P-gp^-ve and P-gp^-ve K562 cells were equally susceptible to lysis over the entire complement concentration range (Fig. 2B, lanes 5–12). Once again, heat inactivation of the rabbit sera inhibited cell lysis (Fig. 2B, lanes 13–16) and only the P-gp^-ve K562 cells were susceptible to drug-induced death (Fig. 2B, lanes 17 and 18).

Complement activation can be inhibited by cell surface regulators of complement activation (RCA) proteins including CD35 (CR1), CD46 (MCP), CD55 (decay accelerating factor), and CD59 (17). To determine whether P-gp^-ve and P-gp^-ve K562 cells expressed equivalent levels of RCA proteins, fluorescence analyses using mAbs specific for the different RCA proteins were performed. As shown in Fig. 3, no significant difference in expression of CD35, CD46, CD55, or CD59 was observed between P-gp^-ve and P-gp^-ve K562 cells.

P-gp Does Not Affect Cell Lysis Induced by Other Pore-forming Proteins—To determine whether P-gp^-ve and P-gp^-ve K562 cells were equally susceptible to death induced by other pore-forming proteins, cells were treated with purified perforin (Fig. 4A) or pneumolysin (Fig. 4B). P-gp did not affect perforin- (Fig. 4A, lanes 1–12) or pneumolysin-mediated cell lysis (Fig. 4B, lanes 1–10), but did protect K562 cells against cell death induced by the chemotherapeutic agents vincristine (Fig. 4A, lanes 13 and 14) and doxorubicin (Fig. 4B, lanes 11 and 12).

**Fig. 8.** RP-gp^-ve cells are sensitive to lysis induced by pore-forming proteins. A and B, ⁵¹Cr-Labeled RP-gp^-ve and RP-gp^-ve cells were incubated with PLO (1.44 µg/ml serially diluted 10-fold) for 4 h at 37 °C (A) or serially diluted rabbit serum containing active complement (C', 1:4–1:64) or heat-inactivated rabbit serum (HI C', 1:4) for 1 h at 37 °C (B). C, to analyze the kinetics of complement-mediated cell lysis, ZVAD-fluoromethyl ketones (Enzyme System Products, Dublin, CA) (final, 50 µM).

Complement-mediated lysis was assessed by treating 5 × 10⁴ ⁵¹Cr-labeled target cells with diluted active or heat-inactivated rabbit complement for 10–60 min at 37 °C in the presence or absence of anti-CD71 (clone M-A712, IgG2a, BD PharMingen) or anti-FcγRII (clone 8.26, IgG2b) (25) monoclonal antibodies where applicable. Lysis by perforin or pneumolysin was assessed by treating 5 × 10⁴ ⁵¹Cr-labeled target cells with diluted pore-forming proteins for 60 min at 37 °C.
These experiments were performed numerous times, and no statistically significant difference in susceptibility to death induced by these agents was observed in P-gp"ve versus P-gp"ve cells. These data therefore demonstrate that functional P-gp does not protect myeloid cells against cell lysis induced by a range of different membranolytic proteins.

**P-gp Does Not Protect T Cell Lines against Membrane Lysis Induced by Different Pore-forming Proteins**—To ensure that the results obtained using the K562 erythoblastoid lines were not specific to myeloid cells, P-gp"ve (A7) and P-gp"ve (CEM) T lymphoid cell lines were used. Cells were treated with activated complement (Fig. 5A), purified perforin (Fig. 5B), and pneumolysin (Fig. 5C) and cell lysis assessed by 51Cr release assays. In all cases, both P-gp"ve and P-gp"ve CEM cells were equivalently susceptible to cell lysis induced by active pore-forming proteins. In contrast, only P-gp"ve CEM cells were susceptible to death induced by chemotherapeutic agents vincristine (Fig. 5, A and B) or doxorubicin (Fig. 5C). It should be noted that the rabbit sera used in this study contained significant levels of natural antibodies against both P-gp"ve and P-gp"ve CEM cell lines as assessed by flow cytometry (data not shown). This thereby eliminated the necessity for anti-CD71 or anti-FcγRII mAbs to be used in the complement activation assays. In addition, there was no significant difference in expression of cell surface CD35, CD46, CD55, and CD59 between parental cells with vincristine and doxorubicin, respectively. It has yet to be identified. Analysis of the results obtained using the K562 erythoblastoid lines were both produced following treatment of parental cells with vincristine and doxorubicin, respectively. It is therefore possible that any affect that P-gp may have on cell lysis induced by complement or other pore-forming proteins may be negated by other genetic defects introduced into these cells following drug treatment. To overcome this potential problem, we expressed P-gp on the surface of CEM cells by retroviral gene transduction in the complete absence of drug selection. CEM cells were transduced with virus expressing GFP and P-gp and were sorted by flow cytometry. Thus, cell lines expressing P-gp and/or GFP were obtained and used in the following studies.

Expression of cell surface P-gp and intracellular GFP in CEM, A7, and retroviral transduced CEM cells was assessed by flow cytometry (Fig. 6A). P-gp was expressed on A7 and fluorescence-activated cell-sorted P-gp"ve (GFP"ve/P-gp"ve) and not on CEM or P-gp"ve (GFP"ve/P-gp"ve) cells (Fig. 6A). The efflux function of P-gp was determined by 125I-Rh efflux assays (Fig. 6B). As 125I-Rh, a demonstrated substrate for P-gp, is a fluorescent dye, an increase in fluorescence following incubation with cells indicates dye uptake (22). Following treatment of cells with 125I-Rh, A7 and RP-gp"ve showed only a marginal increase in fluorescence over background compared with that seen with CEM and RP-gp"ve cells (Fig. 6B). These data indicate that P-gp expressed on the surface of RP-gp"ve cells is capable of effluxing P-gp substrates.

To determine whether P-gp expressed on the retroviral transduced cells confers MDR, the CEM, A7, RP-gp"ve, and RP-gp"ve cells were treated with chemotherapeutic agents vincristine and doxorubicin (Fig. 7). Both A7 and RP-gp"ve cells were resistant to various doses of vincristine and doxorubicin, whereas CEM and RP-gp"ve cells were sensitive to death induced by these agents. These data indicate that RP-gp"ve cells express functional P-gp and that P-gp expression alone is sufficient to induce an MDR phenotype in the CEM T cell line.

We next assessed the sensitivity of RP-gp"ve and RP-gp"ve cells to cell lysis mediated by pneumolysin (Fig. 8A) and complement (Fig. 8B). Both cell lines were equivalently sensitive to death induced by these membranolytic agents, with RP-gp"ve cells demonstrating resistance to death induced by vincristine as a control for P-gp function in these assays (Fig. 8, A and B, lane 14). Both RP-gp"ve and RP-gp"ve cells expressed approximately equal levels of cell surface CD35, CD46, CD55, and CD59 (data not shown). To determine whether RP-gp"ve and RP-gp"ve cells may be lysed by activated complement with different kinetics, time-course experiments were performed. As shown in Fig. 8C, RP-gp"ve and RP-gp"ve cells were lysed by activated complement with very similar kinetics, indicating that, in this *in vitro* system, functional P-gp does not significantly affect the rate of complement deposition.

**DISCUSSION**

The role of P-gp in regulating complement-mediated cell lysis has been a controversial one with different groups reporting conflicting data. One group correlated P-gp expression with increased sensitivity of cells to complement-mediated cell lysis (14). In contrast, two separate studies by another group demonstrated that functional P-gp inhibited the rate of complement deposition on the cell surface and provided evidence suggesting that P-gp inhibited the rate of formation of the complement MAC (15, 16). In an attempt to clarify this issue, we tested a number of different P-gp"ve and P-gp"ve cell lines, including cells transduced with retrovirus and selected in the absence of drug treatment, for sensitivity to cell membrane damage mediated by pore-forming proteins. The retroviral transduced P-gp"ve cells expressed levels of P-gp equivalent to that seen in drug selected CEM-A7 and Kvin cells and were equally insensitive to various chemotherapeutic drugs. This indicates that, in this system, expression of P-gp was necessary and sufficient to induce a multidrug-resistant phenotype. Cell lysis was induced with a diverse range of membranolytic agents including activated complement, purified human perforin, and the bacterial pore-forming protein pneumolysin. Our data indicate that functional P-gp does not significantly affect the rate or degree of membrane lysis irrespective of cell type, the manner by which P-gp expression was achieved, or the lytic agent used.

Although the role of P-gp in effluxing xenotoxins out of cells has long been established, the physiological functions of P-gp have yet to be identified. Analysis of *MDR1* gene knockout mice reveals an inability to clear certain neurotoxins (26, 27), and the mice spontaneously develop ulcerative colitis, possibly the result of an inability to remove bacterial toxins produced by the resident mucosal flora (28). *Using in vitro* systems, we and others have demonstrated additional roles for P-gp including regulation of caspase activation (8, 9), chloride channel activity (29–31), lipid transport (32), and intracellular cholesterol trafficking (33, 34). Expression of P-gp correlates with an increase in intracellular pH (pH1) and decreased plasma membrane potential (*V*max) (35). It has been proposed by Roepe and colleagues (36) that this effect on cell physiology indirectly affects the function of chemotherapeutic drugs that are not directly pumped by P-gp by “partitioning” drugs away from their intracellular targets. As stated above, a role for P-gp in positively or negatively regulating complement-mediated cell lysis has also been proposed. Whether or not any of these proposed roles of P-gp represent true physiological functions of the molecule remains to be thoroughly tested.
expected an increase in complement-mediated lysis or even a zero net effect rather than the decrease in complement activity observed. Interpretation of the results from these studies were made somewhat difficult, given the differences in expression of certain complement regulatory proteins observed between P-gp<i>ve</i> and P-gp<i>ve</i> cells.

The studies by Weisberg and colleagues (15, 16) showing P-gp-mediated resistance to complement-induced cell lysis appeared well controlled, although the relative expression of complement regulatory proteins such as CD35, CD46, CD55, and CD59 on the different P-gp<i>ve</i> and P-gp<i>ve</i> cell lines was not assessed. We attempted to reproduce these findings using the same cell type (K562) and complement activating antibodies directed against the same cell surface antigen (CD71) used in those studies. However, we observed no difference in complement sensitivity between P-gp<i>ve</i> and P-gp<i>ve</i> cells. The cell lines used in our study expressed similar levels of complement regulatory proteins, and the drug efflux function of P-gp was confirmed in every assay. Weisberg <i>et al.</i> argue that altered intracellular pH may be the mechanism by which P-gp can affect complement-induced cell death and hypothesize that other pore-forming proteins such as a complement C9-like protein produced by <i>Trypanosoma cruzi</i> may be similarly affected.

No data have yet been published supporting this hypothesis. In contrast, we demonstrate in our assay systems that neither purified human perforin which is also homologous to complement sensitivity between P-gp <i>ve</i> and P-gp <i>ve</i>, and P-gp-mediated resistance to complement-induced cell lysis appears to be the topic of some debate, and we have attempted to answer this question using a variety of different cell lines expressing P-gp tested against a range of different membranolytic proteins. From our data, we conclude that P-gp does not regulate cell lysis induced by activated complement, purified human perforin, or pneumolysin.

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**REFERENCES**

1. Ueda, K., Cardarelli, C., Gottesman, M. M., and Pastan, I. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 3004–3008

2. Gottesman, M. M., and Pastan, I. (1993) <i>Annu. Rev. Biochem.</i> 62, 385–427

3. Higgins, C. F., and Gottesman, M. M. (1997) <i>Trends Biochem. Sci.</i> 17, 18–21

4. Cordon-Cardo, C., O’Brien, J. P., Boccia, J., Casals, D., Bertina, J. R., and Melamed, M. R. (1990) J. Histochem. Cytochem. 38, 1277–1287

5. Klimecki, W. T., Futschek, B. W., Grogan, T. M., and Dalton, W. S. (1994) <i>Blood</i> 83, 2451–2458

6. Randolph, G. J., Beaulieu, S., Pope, M., Sugawara, I., Hoffman, L., Steinman, R. M., and Muller, W. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6924–6929

7. Robinson, L. J., Roberts, W. K., Ling, T. T., Lamming, D., Sternberg, S. S., and Roepe, P. D. (1997) <i>Biochim. Biophys. Acta</i> 1366, 1169–1178

8. Smyth, M. J., Krassovskis, E., Sutton, V. R., and Johnstone, R. W. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7024–7029

9. Johnstone, R. W., Krassovskis, E., and Smyth, M. J. (1999) <i>Blood</i> 93, 1085–1095

10. Rueff, A. A., Smyth, M. J., and Johnstone, R. W. (2000) <i>Blood</i> 95, 2378–2385

11. Tainton, K. M., Rueff, A. A., Smyth, M. J., and Johnstone, R. W. (2000) <i>Biocomp. Biophys. Res. Commun.</i> 276, 231–237

12. Ahn, C. H., Ahn, Y., Schmidt-Wolf, G., Schmidt-Wolf, I. I., Sikic, B. I., and Chao, N. J. (1991) <i>Blood</i> 77, 2079–2084

13. Tsuruo, T., Hamada, H., Sato, S., and Heike, Y. (1989) <i>Jpn. J. Cancer Res.</i> 80, 627–631

14. Bomstein, Y., and Fishelson, Z. (1997) <i>Europ. J. Immunol.</i> 27, 2204–2211

15. Weisberg, J. H., Curcio, M., Caron, P. C., Raghu, G., Mechetner, E. B., Roepe, P. D., and Scheinberg, D. A. (1996) <i>J. Exp. Med.</i> 183, 2699–2704

16. Weisberg, J. H., Roepe, P. D., Dackenov, S., and Scheinberg, D. A. (1999) <i>J. Biol. Chem.</i> 274, 10877–10888

17. Morgan, P. B., and Harris, C. L. (1999) <i>Complement Regulatory Proteins</i>, pp. 1–31, Academic Press, London

18. Trapani, J. A., Davis, J., Sutton, V. R., and Smyth, M. J. (2000) <i>Curr. Opin. Immunol.</i> 12, 323–329

19. Young, J. D., Cohn, Z. A., and Podack, E. R. (1986) <i>Science</i> 233, 184–190

20. Tschopp, J., Masson, D., and Stanley, K. K. (1986) <i>Nature</i> 322, 831–834

21. Zalberg, J. R., Hu, X. F., Wall, D. M., Miroski, S., Cole, S., Nofalini, G., De Luise, M., Parkin, D. J., Vrazas, V., Campbell, L., and Kanthardip, P. (1994) <i>Int. J. Cancer</i> 57, 522–526

22. Yamamoto, T., Iwashita, T., Watanebe, N., Oshimi, K., Naito, M., Tsuruo, T., and Kobayashi, Y. (1993) <i>Blood</i> 81, 1342–1346

23. Zhou, W., Clouston, D. R., Wang, X., Cerruti, L., Cunningham, J. M., and Jane, S. M. (2000) <i>Mol. Cell. Biol.</i> 20, 7662–7672

24. Friedlich, C. J., Turvey, J., and Hanna, W. (1996) <i>Biochem. Biophys. Res. Commun.</i> 229, 44–49

25. Irico, F., Hulett, M. D., McKenzie, I. F., and Hogarth, P. M. (1993) <i>J. Immunol.</i> 150, 1794–1803

26. Schinkel, A. H., Spits, H., van Tellingen, O., Beijnen, J. H., Wagenaar, E., van Deemter, L., Mol, C. A., van der Valk, M. A., Robanus-Maandag, E. C., te Riele, H. P., and Borst, P. (1994) <i>Cell</i> 77, 491–502

27. Schinkel, A. H., Mayer, U., Wagenaar, E., Mol, C. A., van Deemter, L., Smit, J. J., van der Valk, M. A., Voerdouw, A. C., Spits, H., van Tellingen, O., Zijlmans, J. M., Fibbe, W. E., and Borst, P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4028–4033

28. Panwala, C. M., Jones, J. C., and Viney, J. L. (1998) <i>J. Biol. Chem.</i> 273, 5733–5744

29. Valverde, M., Diaz, M., Sepulveda, F. V., Gill, D. R., Hyde, S. C., and Higgins, C. F. (1992) <i>Nature</i> 355, 830–833

30. Gill, D. R., Hyde, S. C., Higgins, C. F., Valverde, M. A., Mintenig, G. M., and Sepulveda, F. V. (1992) <i>Cell</i> 71, 23–32

31. Hardy, S. P., Goodfellow, N. R., Valverde, M. A., Gill, D. R., Sepulveda, V., and Higgins, C. F. (1995) <i>EMBO J.</i> 14, 68–75

32. van der Valk, P., and van Meer, G. (1996) <i>Nature</i> 381, 68–75

33. Hitch, P., and van der Valk, P., and van Meer, G. (1996) <i>Nature</i> 381, 68–75

34. Luker, G. D., Nilsson, K. R., Covey, D. F., and Piwnica-Worms, D. (1999) <i>J. Biol. Chem.</i> 274, 6979–6991

35. Watkins, R. M., and Roepe, P. D. (1997) <i>Int. Rev. Cytol.</i> 171, 121–165

36. Roepe, P. D. (2000) <i>Curr. Pharm. Des.</i> 6, 241–260

37. Johnstone, R. W., Rueff, A. A., and Smyth, M. J. (2000) <i>Trends Biochem. Sci.</i> 259, 1–6

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