Journal of Bioenergetics and Biomembranes, Vol. 27, No. 1, 1995

Multidrug Resistance and P-glycoproteins in Parasitic Protozoa

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Received January 3, 1995; accepted January 3, 1995

Drug resistance has emerged as a devasting impediment to the treatment and control of diseases of parasitic origin. The underlying mechanisms that contribute to this drug resistance in field isolates, however, are poorly understood. Members of the P-glycoprotein gene (*pgp*) family have been identified, cloned, and sequenced in *Plasmodia*, *Leishmania*, and *Entamoeba*, and variations in *pgp* copy number and/or expression have been implicated as a basis for drug resistance in each of these genera. The spectrum of drugs to which parasitic protozoa containing amplified *pgp* genes and/or transcripts are refractory range from a phenotype similar to that observed with multidrug-resistant mammalian cells to those that are completely distinct. The availability of molecular probes to *pgp* genes provides valuable reagents to dissect the role of *pgp* gene amplification and overexpression in mediating drug resistance in parasitic protozoa and to determine the physiological function of P-glycoproteins in this clinically consequential group of human pathogens.

KEY WORDS: P-glycoproteins; multidrug resistance; parasites; protozoa; *Plasmodium*; *Leishmania*; *Entamoeba*.

INTRODUCTION

Parasitic protozoa are the causative agents of a plethora of devastating and often fatal diseases in humans. Diseases of protozoan parasites that produce significant morbidity and mortality include malaria, African sleeping sickness, Chagas disease, leishmaniasis, amoebic dysentery, giardiasis, cryptosporidiosis, and toxoplasmosis. In fact, afflictions of parasitic etiology probably constitute the most significant assortment of diseases worldwide in terms of human misery and economic spoliation. These single-cell eukaryotic pathogens have evolved complex life cycles often involving intermediate hosts and exhibit an unusual and fascinating assortment of molecular, biochemical, metabolic, and structural oddities that distinguish them from other eukaryotes. Chemotherapeutic regimens for treating or averting parasitic diseases have been by-and-large derived empirically and are not particularly selective for the metabolic machinery of the parasite. Unsurprisingly, therefore, antiparasitic drugs are noted for causing moderate to severe toxicity in the human host.

A major obstacle to the treatment, prevention, and eradication of parasitic diseases in humans has been and continues to be drug resistance, a problem that has been particularly identified with malaria and African sleeping sickness but has materialized as a serious issue with other parasitic diseases as well (Geary *et al.*, 1986). This emergence of drug resistance in parasitic diseases can be ascribed, at least in part, to noncompliance or discontinuation of therapy prompted by the deleterious side effects of the drug regimens. Almost nothing is known about the biochemical alterations or molecular mechanisms by which parasites become resistant or refractory to drugs in the field, and only recently have biochemical and molecular biological techniques been applied to analyze the events that precipitate drug resistance in these pathogens. Knowledge of the molecular phenomena that underlie drug resistance in parasitic protozoa may disclose avenues which either circumvent the problems associated with the development of drug resistance or preclude the phenomenon from

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0145-479X/95/0200-0077$07.50/0

occurring altogether. That clinical unresponsiveness to chemotherapeutica may not always reflect true organismal drug resistance but rather genetic, immunologic, or physiological factors in the infected host should also be acknowledged.

The role of P-glycoproteins (PGPs) in mediating drug resistance in parasitic protozoa has gained considerable attention within the parasitology community. This interest arose initially from apparent similarities in the phenotypes of chloroquine-resistance (CQR) isolates of *Plasmodium falciparum*, some of which are resistant to several antimalarial drugs, and multidrug-resistant (MDR) mammalian cells. Genes encoding members of the PGP family (*pgp*) have now been isolated and analyzed from *Plasmodia*, *Leishmania*, and *Entamoeba*, and variations in *pgp* copy number and/or levels of expression have been implicated in drug resistance in all three genera. A gene encoding a PGP-like half molecule has also been cloned from *Trichomonas vaginitis*, but its role in mediating drug resistance is questionable. Changes in the number of *pgp* genes have been associated with differential susceptibilities of *P. falciparum* to chloroquine (CQ) and mefloquine among field isolates, with MDR phenotypes in cultured forms of *Plasmodia*, *Leishmania*, and *Entamoeba*, and with some drug-refractory isolates of *T. vaginitis*. The physiological functions of parasite *pgp* genes and PGP proteins are unknown. It is the intent of this commentary to review the available molecular and biochemical data pertaining to *pgp* genes and their encoded products in parasitic protozoa and to examine the role of *pgp* amplification and expression in conferring drug resistance in these organisms.

P-GLYCOPROTEIN GENES AND DRUG RESISTANCE IN *Plasmodium falciparum*

Resistance of malaria to CQ is found throughout endemic regions of the world and has enormous and oppressive clinical implications. This CQ resistance is primarily associated with *P. falciparum*, the most virulent and dangerous form of the malarial parasite, and has prompted the use of less efficacious and more expensive drugs to treat the disease. However, resistance to these alternative drugs, such as mefloquine, pyrimethamine, and cycloguanil, has also been widely reported (Geary *et al.*, 1986). In fact, resistance to multiple antimalarials has been observed in both clinical isolates (Webster *et al.*, 1985; Draper *et al.*, 1988; Gay *et al.*, 1990; Wilson *et al.*, 1993) and in drug-selected organisms *in vitro* (Odoula *et al.*, 1988; Cowman *et al.*, 1994). In some *P. falciparum* strains, CQ resistance has been attributed to diminished drug accumulation or elevated drug efflux (Verdier *et al.*, 1985; Krogstad and Schlesinger, 1987; Krogstad *et al.*, 1987). Moreover, several reports have indicated that both CQ resistance and diminished drug accumulation can be reversed by the addition of verapamil (Martin *et al.*, 1987) or desipramine (Bitonti *et al.*, 1988), calcium channel antagonists that can ameliorate the MDR phenotypes of mammalian cells (Gottesmann and Pastan, 1988).

The conspicuous similitude between the growth phenotypes of drug-resistance *P. falciparum* and MDR mammalian cells stimulated a search for MDR gene (*mdr*) homologs within the *P. falciparum* genome (Foote *et al.*, 1989; Wilson *et al.*, 1989). Degenerate oligonucleotides were constructed to the two nucleotide binding motifs in the mammalian *mdr1* gene using the known codon bias of plasmodial protein coding genes, and fragments of two genes, designated *pfmdr1* and *pfmdr2*, were amplified from *P. falciparum* genomic DNA using the polymerase chain reaction (PCR) and employed as probes to clone full-length *pfmdr1* (Foote *et al.*, 1989) and *pfmdr2* (Zalis *et al.*, 1993; Rubio and Cowman, 1994). The size, amino acid sequence, and predicted membrane topology of the *pfmdr1* gene product, designated PGH1 by workers in the field, was typical of members of the PGP family (Foote *et al.*, 1989) (Table I). PGH1 consisted of two homologous halves separated by a hydrophilic, asparagine-rich hinge region. Each half-molecule was composed of six putative transmembrane domains followed by a hydrophilic region containing a nucleotide binding motif. Unsurprisingly, the two nucleotide binding sites exhibited the greatest degree of homology with other PGPs. A pairwise alignment between the human *mdr1* amino acid sequence and PGH1 revealed a 33% identity (Table I). Each half of PGH1 exhibited greater sequence homology to its mammalian counterpart than to its partner, indicating that the ancestral gene duplication and subsequent gene fusion events that have been postulated for many members of the PGP family occurred prior to the phylogenetic separation of *Plasmodia* from other members of the eukaryotic lineage (Foote *et al.*, 1989). Biochemical studies have revealed that PGH1 exists as a serine- or threonine-phosphoprotein in all life cycle stages and that this phosphorylation cannot

Table I. Structural Properties of Parasite PGPs

Organism	*pgp* gene	Molecular mass (dK)	Number of amino acids	Homologous halves	Number of transmembrane domains	Number of ATP binding sites	Amino acid identity with human *mdr1* PGP (%)
P. falciparum	*pfmdr1*	162	1419	Yes	12	2	33
	pfmdr2	119	1025	No	10	1	26
Leishmania	*ltpgpA*	172	1548	Yes	12	2	22
	ldmdr1	147	1341	Yes	12	2	37
E. histolytica	*EhPgp1*	145	1302	Yes	12	2	41
	EhPgp2	145	1310	Yes	12	2	40
	Ehabc1	92	808	No	5	1	n.d.
T. vaginalis	*Tvpgp1*	65	589	No	6	1	32–35[a]

[a] *Tvpgp1* is equivalent to half of a full length PGP. The *Tvpgp1* gene product is 34.5% and 32.2% identical to the NH_2-terminal and COOH-terminal halves of the human *mdr1* gene product, respectively.

be reduced by CQ or modulators of CQ resistance (Lim and Cowman, 1993). Moreover, the protein can be photoaffinity labeled with 8-azido-ATP, consistent with a presumed nucleotide binding function for PGH1 *in vivo* (Karcz *et al.*, 1993).

Analysis of the somewhat shorter *pfmdr2* protein (Zalis *et al.*, 1993; Rubio and Cowman, 1994) predicted a somewhat unusual membrane topology for a member of the PGP family, one consisting of 10 membrane spanning regions followed by a single COOH-terminal nucleotide binding site (Table I). The *pfmdr2* and human *mdr1* gene products exhibited only a 26% amino acid identity with one another (Table I). The primary structure and atypical topology of the *pfmdr2* amino acid sequence is most closely identified with that of the cadmium resistance gene of *Schizosaccharomyces pombe* (Ortiz *et al.*, 1992). Interestingly, *pfmdr2* appears to be expressed to a much greater degree in trophozoites and schizonts than in ring-state parasites (Zalis *et al.*, 1993; Rubio and Cowman, 1994), whereas *pfmdr1* is expressed throughout the erythrocytic phase of the malarial life cycle (Foote *et al.*, 1989), although one of the two *pfmdr1* mRNAs, the 7.5-kb transcript, is exclusively expressed in the trophozoite stage of the parasite (Volkman *et al.*, 1993).

The role of *pfmdr1* and *pfmdr2* in mediating susceptibility of *P. falciparum* to drugs has stimulated considerable investigation. Initial reports indicated that amplification, overexpression, and the presence of specific *pfmdr1* alleles were preferentially associated with some CQR strains of *P. falciparum* (Foote *et al.*, 1989, 1990). Restriction mapping indicated that the *pfmdr1* amplicon was organized in several CQR isolates as a tandemly repeated array on the chromosome on which the wild type *pfmdr1* locus is located (Foote *et al.*, 1989), and PCR experiments indicated that these *pfmdr1* amplifications arose from multiple independent events (Triglia *et al.*, 1991). The linkage of *pfmdr1* expression with CQ resistance was bolstered by immunofluorescence and immunoelectron microscopy data that revealed that PGH1 is localized to the membrane of the parasite food vacuole (Cowman *et al.*, 1991), the organelle in which CQ accumulates (Krogstad *et al.*, 1985; Krogstad and Schlesinger, 1987).

The initial association of *pfmdr1* amplification, overexpression, or certain intragenic alleles with CQ resistance in malaria, however, has not been sustained by subsequent findings. First, phenotypic dissection of recombinant progeny between clonal isolates of CQ-sensitive and CQR *P. falciparum* established that neither the drug resistance nor the rapid drug efflux phenotype of the resistant strains cosegregated with either *pfmdr1* or *pfmdr2* or their amplification (Wellems *et al.*, 1990). Second, the CQ resistance locus in the above cross was mapped to chromosome 7 (Wellems *et al.*, 1991), rather than to chromosome 5 on which *pfmdr1* is located (Foote *et al.*, 1989). Third, sequence analysis of a number of malarial isolates from Thailand revealed no connection between certain alleles of *pfmdr1* and CQ resistance (Wilson *et al.*, 1993). Fourth, selection of several CQR isolates with amplified *pfmdr1* copies in higher CQ concentrations resulted in deamplification and decreased expression of *pfmdr1* (Barnes *et al.*, 1992). Moreover, verapamil reversed this high CQ resistance. These data suggest that high levels of CQ resistance are not mediated by augmented PGH1 expression. Surprisingly, an increased sensitivity to mefloquine

was observed in all three cell lines selected for high CQ resistance (Barnes *et al.*, 1992). Fifth, a *pfmdr1* gene from a CQ-sensitive isolate was expressed in Chinese hamster ovary (CHO) cells and shown to confer increased susceptibility to CQ toxicity and an enhanced ATP-dependent CQ uptake capacity (Van Es *et al.*, 1994). Two *pfmdr1* alleles from different CQR strains were also transfected into CHO cells, but their expression did not augment CQ growth sensitivity or incorporation over background. These data are consistent with those obtained from the *P. falciparum* strains selected for high CQ resistance and imply that PGH1 is involved in CQ import into the food vacuole, rather than CQ efflux, and that *pfmdr1* amplification and overexpression increases CQ susceptibility. Finally, the initial interpretation that CQ efflux is a cause of CQR in *P. falciparum* (Krogstad *et al.*, 1987) has been strongly disputed by subsequent kinetic appraisal of the available data (Ferrari and Cutler, 1991; Ginsburg and Stein, 1991) and by additional kinetic studies (Bray *et al.*, 1992).

Mefloquine resistance, conversely, does appear to be coupled to *pfmdr1* amplification and overexpression. Wilson *et al.* (1993) have established a solid connection between *pfmdr1* amplification and mefloquine, as well as halofantrine, resistance in 11 field isolates of *P. falciparum*. Moreover, *in vitro* selection of two CQR *P. falciparum* strains for mefloquine resistance resulted in *pfmdr1* amplification and overexpression, cross resistance to both halofantrine and quinine, and a diminution in CQ resistance (Cowman *et al.*, 1994). Thus, it appears that PGH1 expression is associated with a MDR phenotype involving mefloquine but with CQ susceptibility (Table II). Despite the connection of *pfmdr1* with drug resistance in *P. falciparum*, amplification and overexpression of *pfmdr2* has never been observed among *P. falciparum* populations (Wilson *et al.*, 1989; Zalis *et al.*, 1993; Rubio and Cowman, 1994).

P-GLYCOPROTEIN GENES AND DRUG RESISTANCE IN *Leishmania*

Leishmania species cause a spectrum of diseases ranging from a self-limited cutaneous infection to the virulent and usually fatal visceral disease. Resistance to pentavalent antimonials, the first-line antileishmanial drugs, is a frequent occurrence that develops in 5–70% of afflicted individuals in endemic regions (Jackson *et al.*, Grogl *et al.*, 1992). Unlike for drug-resistant malaria, there is essentially no information on the biochemical mechanisms that underlie antimonial resistance in field isolates of this parasite. However, the amenability of cultured *Leishmania* promastigotes to genetic manipulation has made this genus the focus of numerous studies on drug resistance in parasitic protozoa (Ouellette and Papadopoulou), 1993), among which are several that implicate *pgp* genes and their amplification in this insusceptibility. Two classes of *pgp* genes have been described in *Leishmania spp.*

The first class of *pgp* genes, represented by the now well-characterized *ltpgpA* and *lmpgpA* genes from *L. tarentolae* and *L. major*, respectively, was discovered (Ouellette *et al.*, 1990) on an extrachromosomal element that is frequently amplified after selection of wild type parasites with a hydrophilic drug. This small circular DNA, designated the H circle, is observed in *Leishmania spp.* selected for resistance to one of a variety of structurally and mechanistically unrelated drugs, including methotrexate, arsenite, primaquine, and terbinafine, and these mutant derivatives are often MDR (Ellenberger and Beverley, 1989; Katakura and Chang, 1989; Ouellette *et al.*, 1990,

Table II. Drug Resistance Phenotypes Associated with *pgp* Overexpression

Gene	Drugs
pfmdr1	Mefloquine
	Halofantrine
	Quinine
pfmdr2	None
ltpgpA	Aresnite
lmpgpA	Antimonite
	Pentostam[b]
ldmdr1	Vinblastine
lemdr1	Puromycin
	Anthracyclines[c]
Ehpgp[d]	Emetine
	Colchicine
	Iodoquinol[e]
	Diloxanide[e]
Tvpgp1	Metronidazole[f]

[a] *pfmdr1* overexpression correlated with increased chloroquine susceptibility.

[b] *ltpgpA*, but not *lmpgpA*, overexpression associated with Pentostam resistance.

[c] Anthracyclines only tested with *ldmdr1* overexpressors.

[d] Which of the several *E. histolytica pgp* genes is responsible for bestowing drug resistance is unknown.

[e] Data for iodoquinol and diloxanide have not been reported.

[f] Three of seven metronidazole-resistant strains of *E. histolytica* overexpress *Tvpgp1*.

1991). At least four other *pgp* genes of this class have now been identified in *L. tarentolae*, *ltpgpB*, *ltpgpC*, *ltpgpD*, and *ltpgpE*, all of which hybridize to a *ltpgpA*-derived probe at high stringency (Ouellette *et al.*, 1990, 1991; Gamarro *et al.*, 1994). Sequence analysis of *ltpgpA* and hydropathy plots predict a pattern typical of PGP proteins, two homologous halves, each consisting of six transmembrane segments followed by a nucleotide binding site (Table I). The LTPGPA bears greatest similarity to the MDR-associated protein from MDR lung cancer cells (Cole *et al.*, 1992) and is only 22% identical with the human *mdr1* protein (Table I).

The phenotypes of *Leishmania* containing amplified H circle were dissimilar to those of MDR mammalian cells, as the MDR parasites were sensitive to hydrophobic agents that are substrates for the mammalian MDR protein (Ellenberger and Beverley, 1989; Ouellette *et al.*, 1990, 1991). The complex phenotype of these parasites could be attributed, in part, to the presence of an additional gene on the H circle, terms *ltdh* in *L. tarentolae* (Papadopoulo *et al.*, 1992) and *ptr1* in *L. major* (Callahan and Beverley, 1992; Bello *et al.*, 1994). *ltdh* and *ptr1* encode a pteridine reductase activity that mediates the resistance to methotrexate (Bello *et al.*, 1994). The relative contribution of *pgpA* to the complex drug resistance phenotype observed in MDR *Leishmania* was dissected by transfection studies. This analysis showed that *pgpA* confers low levels of resistance to arsenite and antimonite, but not to other metals, vinblastine, or puromycin, in *L. tarentolae* and *L. major* (Callahan and Beverley, 1991; Papadopoulou *et al.*, 1994). This *pgpA*-mediated metal oxyanion resistance, unlike mammalian MDR or malarial CQ resistance, is not reversed by verapamil. No differences in arsenite uptake were observed in the *ltpgpA L. tarentolae* transfectants (Papadopoulou *et al.*, 1992), although *lmpgpA*-transfected *L. major* accumulated considerably less arsenite than wild type or mock transfected controls (Callahan *et al.*, 1994). This discrepancy can be attributed to the fact that *ltpgpA* transfection accords only a 2-fold resistance to arsenite, whereas transfection of *lmpgpA* augments decreases arsenite sensitivity by an order of magnitude. The *lmpgpA*-mediated reduction in arsenite uptake can be ascribed to a decreased influx rather than an augmented efflux mechanism (Callahan *et al.*, 1994). Thus, *pgpA* appears to mediate, like the malarial PGH1, ligand influx rather than efflux.

The role, if any, of *pgp* genes in mediating resistance to Pentostam, the most commonly used antileishmanial, is not clear. Interestingly, transfection of *ltpgpA* into wild type *L. tarentolae* triggered resistance to Pentostam (Papadopoulou *et al.*, 1992), whereas transfection of *lmpgpA* into *L. major* did not (Callahan and Beverley, 1991), a discrepancy that could perhaps be attributed to allelic differences. It should be noted that diminished Pentostam accumulation has been observed in Pentostam-resistant *Leishmania* and that these resistant strains appear to express some protein that cross reacts with a monoclonal antibody to the mammalian MDR protein (Grogl *et al.*, 1991). Whether this overexpressed protein is related to the *pgpA* gene product or any other member of the PGP family is dubious.

Recently, a novel MDR strain of *L. tropica* has been selected on methotrexate that overexpresses *ltpgpE* (Gamarro *et al.*, 1994). This overexpression is correlated with a rearrangement of the *ltpgpE* gene rather than *ltpgpE* amplification. The mutant *L. tropica* possesses a novel MDR phenotype involving low-level resistance to arsenite, vinblastine, puromycin, and doxorubicin. Overexpression of *ltpgpB*, *ltpgpC*, and *ltpgpD* has not been correlated with any observed differences in drug susceptibility.

The second class of *pgp* genes that have been identified and characterized in *Leishmania* species are those that confer phenotypes similar to those of MDR mammalian cells. Employing the same PCR-based approach used to isolate fragments of the malarial *pfmdr1* and *pfmdr2* genes, Wirth's group amplified a number of *pgp* fragments from *L. enriettii* genomic DNA (Henderson *et al.*, 1992). One of these *pgp* probes, LEMDR06, was then used to isolate full-length equivalents in *L. donovani* (Hendrickson *et al.*, 1993) and *L. enriettii* (Chow *et al.*, 1993). Pairwise alignments of LDMDR1 and LEMDR1 with their mammalian *mdr1* protein counterparts revealed a 37% amino acid sequence identity, whereas the *ltpgpA* PGP was identical to the human *mdr1* PGP at only 22% of the amino acid positions (Table I). The *ldmdr1* and *lemdr1* gene products are 83% identical. In fact, comparisons of LDMDR1 to all sequences in the GenBank database indicated quite clearly that LDMDR1 bears by far the greatest resemblance to the mammalian *mdr1*, *mdr2*, and *mdr3* gene products than to other known members of the PGP family (Hendrickson *et al.*, 1993). Hydropathy plots indicated that both proteins exhibit the classic PGP membrane topology, specifically 12 transmembrane domains and two ATP binding sites, arranged as two homologous halves (Table I).

Both *ldmdr1* and *lemdre1* were amplified and overexpressed in vinblastine-selected lines of *L. donovani* (Henderson *et al.*, 1992) and *L. enriettii* (Chow *et al.*, 1993), respectively. The vinblastine-resistant strains displayed cross resistance to puromycin, and the VINB1000 *L. donovani* strain was also less sensitive to the anthracyclines. The MDR phenotype of these vinblastine-derived lines paralleled that of many MDR mammalian cell lines, except that the parasite drug resistance was refractory to verapamil. The VINB1000 *L. donovani* failed to accumulate radiolabeled puromycin, but whether the accumulation defect can be imputed to influx or efflux defects was not ascertained for technical reasons (Henderson *et al.*, 1992). Conversely, viable null mutants in which the *lemdr1* locus has been deleted by homologous gene replacement exhibited a 4-fold increase in vinblastine susceptibility (Chow and Wirth, 1994). This implies that *lemdr1* is not essential for viability of *L. enriettii* promastigotes in culture. Transfection of the *ldmdr1* gene into wild type *L. donovani* conferred the same drug resistance phenotype as the MDR parasites created by drug selection, whereas transfection of *lemdr1* accorded only a partial resistance phenotype. Thus, phenotypic analysis of *Leishmania* in which the *mdr1* gene is amplified and overexpressed after either selection or transfection, coupled with the extensive amino acid sequence homology to the mammalian *mdr* genes, strongly intimates that *ldmdr1* and *lemdr1* are homologs of the mammalian *mdr* genes.

Nothing is known about the physiological function of the *ldmdr1* and *lemdr1* genes in *Leishmania*. Moreover, wild type promastigotes of *L. enriettii* (Chow *et al.*, 1993) and *L. donovani* (Henderson *et al.*, 1992) expressed little to no detectable amounts of the corresponding transcripts. Recently, it has been shown that *lemdr1* could complement the mating type defect in *ste6* yeast, suggesting that some lipophilic peptides are ligands for the protein (Volkman *et al.*, 1994). However, the physiological ligands for this PGP have not been discerned, and a role for *mdr1* in Pentostam resistance in *Leishmania* is unlikely given the charged nature of the antileishmanial drug.

P-GLYCOPROTEIN GENES AND DRUG RESISTANCE IN *Entamoeba histolytica*

E. histolytica, a protozoan parasite that causes millions of cases of dysentery and liver abscesses worldwide, is another genus of parasite in which *pgp* genes have been identified. The *E. histolytica* genome contains at least six *pgp* genes, four complete genes and two pseudogenes, as well as a gene, *Ehabc1*, encoding a novel member of the ATP binding cassette family (Descoteaux *et al.*, 1992; Zhang and Samuelson, 1993). Two of the complete *pgp* genes have been sequenced and shown to exhibit 40–41% positional identity with the human MDR1 protein (Table I). Hydropathy plots reveal the archetypal PGP membrane topology of two alike halves, each consisting of six putative membrane spanning domains followed by a nucleotide binding motif (Descoteaux *et al.*, 1992). *Ehabc1* has also been sequenced and encodes a protein of 91.7 kD with at least five conjectured transmembrane segments followed by a single ATP binding site (Table I). The *Ehabc1* gene product is most homologous to the bacterial permeases (Zhang and Samuelson, 1993).

An emetine-resistant clone of *E. histolytica* has been generated that overproduces *pgp* mRNAs, but which *pgp* transcript is overexpressed has not been determined (Samuelson *et al.*, 1990). Interestingly, this overexpression of *pgp* mRNAs is not coincident with *pgp* gene amplification. The emetine-resistant clone exhibits an MDR phenotype that includes resistance to several other antiamebic drugs including colchicine, iodoquinol, and diloxanide (Table II), and the emetine-resistance is reversible with calcium channel blockers (Orozco *et al.*, 1985; Ayala *et al.*, 1990; Samuelson *et al.*, 1990; Descoteaux *et al.*, 1992). Increased efflux and decreased accumulation of emetine have also been mentioned (Descoteaux *et al.*, 1992). Thus, the MDR phenotype of the emetine-selected derivative of *E. histolytica*, like the MDR phenotype to hydrophobic drugs described in *Leishmania*, parallels that of MDR mammalian cells.

A P-GLYCOPROTEIN-LIKE GENE IN *Trichomonas vaginalis*

A gene encoding a PGP-like protein, *Tvpgp1*, has recently been isolated and characterized from *T. vaginalis* (Johnson *et al.*, 1994), a protozoan parasite that is a common cause of vaginitis that can be treated with metronidazole with varying success. This PGP-like protein contains only 589 amino acids and has six postulated membrane-spanning domains and one ATP binding site (Table I). The *Tvpgp1* gene product is 34.5% and 32.2% identical with the NH_2- and

COOH-terminal domains of the human *mdr1* gene product, respectively. The *Tvpgp1* gene is overexpressed in three of seven metronidazole-resistant strains, although there is no correlation between the extent of *Tvpgp1* expression and drug resistance (Table II). The gene, however, is not amplified in the metronidazole-resistant isolates. The fact that this PGP-like molecule is not tandemly duplicated suggests the possibility that the tandem duplication and subsequent gene fusion events that created the large PGP molecules in other species occurred very early in eukaryotic evolution, subsequent to the divergence of trichomonads but prior to the separation of kinetoplastids from the eukaryotic lineage (Johnson *et al.*, 1994; Sogin *et al.*, 1986).

CONCLUSIONS

The genomes of parasitic protozoa encompass members of the *pgp* family, and two *pgp* genes each have been cloned, sequenced, and characterized from *Plasmodia*, *Leishmania*, and *Entamoeba*. Amplification and overexpression of *pgp* genes in model systems *in vitro* can effect resistance to certain drugs, as well as MDR phenotypes, but the spectrum of agents to which these parasites are refractory extends from hydrophilic to hydrophobic drugs. Moreover, the copy number and magnitude of expression of the malarial *pfmdr1* gene appears to influence susceptibility of field isolates to several prominent malarial agents, including CQ and mefloquine. Parasites overexpressing *pgp* genes accumulate drugs inefficiently, but this diminished uptake capacity has never been definitively imputed to an impaired efflux mechanism. At this time, neither the naturally occurring ligands nor the physiological functions of parasite PGP proteins have been identified. It can be speculated that PGPs are involved either in the import of important nutritional molecules from the extracellular milieu into the parasite or in the export of toxic compounds from within the organism.

ACKNOWLEDGMENTS

This work was supported by Grant AI32036 from the National Institute of Allergy and Infectious Diseases. B.U. is a Burroughs Wellcome Fund Scholar in Molecular Parasitology, and this work was supported in part by a grant from the Burroughs Wellcome Fund.

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