Schistosome ABC multidrug transporters: From pharmacology to physiology

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Abstract

Praziquantel (PZQ) is essentially the only drug currently available for treatment and control of schistosomiasis, a disease affecting hundreds of millions worldwide. Though highly effective overall, PZQ has limitations, most notably its significant lack of activity against immature schistosomes. Furthermore, the availability of only a single drug for a disease of this magnitude makes reports of PZQ-resistant isolates particularly troubling. ATP-binding cassette (ABC) multidrug transporters such as P-glycoprotein (Pgp; ABCB1) are efflux transporters that underlie multidrug resistance (MDR); changes in their expression or structure are also associated with drug resistance in parasites, including helminths. This review will discuss the role these transporters might play in modulating schistosome susceptibility to PZQ, and the implications for developing new or repurposed treatments that enhance the efficacy of PZQ. However, in addition to influencing drug susceptibility, ABC transporters play important roles in several critical physiological functions such as excretion and maintenance of permeability barriers. They also transport signaling molecules with high affinity, and several lines of evidence implicate mammalian transporters in a diverse array of physiological functions, including regulation of immune responses. Like their mammalian counterparts, schistosome ABC transporters appear to be involved in functions critical to the parasite, including excretory activity and reproduction, and we hypothesize that they underlie at least some aspects of parasite–host interactions. Thus, in addition to their potential as targets for enhancers of PZQ susceptibility, these transporters might also serve as candidate targets for agents that disrupt the parasite life cycle and act as antischistosomals on their own.

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1. Introduction

Efflux transporters of the ATP binding cassette (ABC) protein superfamily mediate multidrug resistance (MDR), and are also associated with drug resistance in parasites, including helminths (reviewed in Leprohon et al., 2011; Kasinathan and Greenberg, 2012; Lespine et al., 2012; Ardelli, 2013; Greenberg, 2013a). As ABC transporters can serve as important regulators of drug susceptibility, they are excellent candidate targets for inhibitors that act as adjuncts to current anthelmintics to enhance effective potency. Indeed, several laboratories have been exploring the possibility of increasing anthelmintic effectiveness and overcoming drug resistance by repurposing currently-available drugs that can inhibit ABC transporters. This exciting approach, which could provide new strategies for combination therapy, has recently been reviewed extensively (James et al., 2009a,b; Ardelli, 2013; Greenberg, 2013a,b; Lespine et al., 2012). This review will also briefly discuss this strategy, but will focus primarily on the possible physiological functions of ABC transporters within schistosomes, and the potential for their exploitation as therapeutic targets on their own.

2. Schistosomes, schistosomiasis, and the need for new therapeutics

Schistosomiasis, caused by parasitic flatworms of the genus Schistosoma, is a major neglected tropical disease that constitutes a significant health and economic burden for hundreds of millions of people (van der Werf et al., 2003; King, 2010). Schistosome infections can produce severe damage to various organs, significant morbidity, impair childhood development and adult productivity, potentially increase susceptibility to other infections such as HIV, and, in some cases, lead to death (van der Werf et al., 2003; King and Dangerfield-Cha, 2008; Hotez and Fenwick, 2009: King, 2010; Ndeffo Mbah et al., 2013; Colley et al., 2014). There is at present no vaccine. Infrastructural and educational interventions to reduce transmission and prevent infection can be highly effective (Tanaka and Tsuji, 1997), but are expensive and require levels of organization that are often impractical in the developing world. Use of mollusccides to eliminate the intermediate host snails raises environmental concerns, can be expensive, and often produces only limited, short-term effectiveness (Sturrock, 2001).

For the past several years, both treatment and control of schistosomiasis have relied on chemotherapy with praziquantel (PZQ), the current drug of choice and essentially the only antischistosomal drug commercially available. Dependence on a single drug would be inadvisable for any infectious disease, but is particularly troubling for one at such high prevalence (Caffrey, 2007). PZQ has been available for several decades, and the history of its development and use has been reviewed extensively (Andrews et al., 1983; Redman et al., 1996; Cioli and Pica-Mattoccia, 2003; Doenhoff and Pica-Mattoccia, 2006; Doenhoff et al., 2008; Chai, 2013; Cioli et al., 2014). The main advantages of PZQ include its effectiveness against all human schistosome species, its comparatively mild side effects, and, in more recent years, its relatively low cost (Ndeffo Mbah et al., 2013). Large-scale PZQ treatment programs have produced significant reductions in disease prevalence and intensity (Veinervald et al., 2005; Toure et al., 2008; Sesay et al., 2014); indeed, based on this success, Merck has committed to donating 250 million tablets of PZQ annually for mass drug administration by 2016. Nonetheless, PZQ does have significant shortcomings. Most notably, schistosomes show major stage-specific differences in PZQ susceptibility: immature worms (2–4 weeks post infection) exhibit insusceptibility to PZQ, making treatment largely ineffective until egg production begins approximately 5–6 weeks post-infection (Xiao et al., 1985; Sabah et al., 1986; Pica-Mattoccia and Cioli, 2004; Aragon et al., 2009). Though typical failure rates for PZQ are in the 5–30% range, certain regions and age groups show lower levels of efficacy, with failure rates often reaching 30–50% (Day and Botros, 2006; King et al., 2011; Mutapi et al., 2011; Sousa-Figueiredo et al., 2012; Garba et al., 2013; Tukahewanu et al., 2013). Furthermore, several field isolates exhibit further reduced susceptibility to PZQ, and PZQ resistance can be experimentally induced in schistosomes (reviewed by Day and Botros, 2006; Doenhoff and Pica-Mattoccia, 2006; Wang et al., 2012; Greenberg, 2013b). Though there is at present no credible evidence for emergence of widespread PZQ resistance, it remains a distinct possibility, particularly as mass drug administration programs are intensified. Furthermore, the mode of action of PZQ has yet to be defined rigorously. PZQ disrupts Ca^{2+} homeostasis in the parasite, with resultant paralysis and tegumental disruption, and several lines of evidence point to voltage-gated Ca^{2+} channels as important mediators of PZQ activity (Greenberg, 2005a; Chan et al., 2012, 2014). Nonetheless, the precise identity of the primary “PZQ receptor” remains unresolved (Redman et al., 1996; Doenhoff et al., 2008; Cioli et al., 2014), confounding rational approaches that might target similar pathways for increased potency or to overcome drug resistance.

Defining the molecular target of PZQ is obviously essential, but will likely not fully illuminate how PZQ works. Thus, several lines of evidence point to a role for downstream factors that influence parasite susceptibility to the drug. The most obvious example can be found in the difference in PZQ responsiveness between adult and juvenile worms. As in adults, PZQ induces a rapid, Ca^{2+}-dependent contraction and paralysis of juvenile worms (Pica-Mattoccia et al., 2008). However, unlike adult worms, which remain paralyzed and ultimately die, the juveniles recover. This remarkable phenomenon suggests that though adults and juveniles share the PZQ molecular target and initial outputs (e.g., Ca^{2+} influx, perhaps via Ca^{2+} channels, contraction and paralysis); juveniles, but not adults, can marshal downstream protective responses that allow them to survive and recover. Indeed, gene expression profiling of adult and juvenile worms following exposure to PZQ supports the notion that these developmental stages differ in the way they respond to the drug (Hines-Kay et al., 2012).

As we and others have discussed, combination therapy, in which current anthelmintics are potentiated by new or repurposed agents that target different, but possibly interacting, sites of action, represents a potentially powerful strategy for overcoming drug resistance and enhancing drug efficacy (Geary et al., 2010; Hu et al., 2010; Greenberg, 2013b). P-glycoprotein (Pgp) and other ATP binding cassette (ABC) efflux transporters have been proposed as particularly attractive targets for this type of approach (Liang and Aszalos, 2006; Lespine et al., 2008). Several recent reviews have summarized the evidence that ABC multidrug transporters modulate helminth drug susceptibility (and resistance) and have explored their potential as targets to enhance anthelmintic activity (Leprohon et al., 2011; Kasinathan and Greenberg, 2012; Lespine et al., 2012; Ardelli, 2013; Greenberg, 2013a). What is particularly appealing about such a strategy is that many inexpensive drugs already in clinical use are known to interact with (i.e., inhibit) these transporters, providing the potential for repurposing these compounds for use in an anthelmintic treatment and control strategy. This type of approach is under intensive investigation in several labs.

On the other hand, ABC transporters are part of an ancient superfamily of proteins, and clearly have physiological functions beyond regulating susceptibility to modern drugs. This review will focus on those possible functions in schistosomes, with an eye towards how they may be exploited to disrupt the parasite life cycle or reduce disease pathology and transmission.
3. ABC transporters

ABC transporters are members of the ABC protein superfamily, a large, ancient group of proteins with representation in all kingdoms of life (Dassa and Bouige, 2001; Borst and Elferink, 2002). The common defining feature of ABC transporters is that they contain nucleotide binding domains (NBDs) that bind ATP (the ATP binding cassettes), and use the energy resulting from ATP hydrolysis to translocate compounds across the membrane. Some prokaryotic ABC transporters import compounds into the cell; efflux ABC transporters are found in both prokaryotes and eukaryotes (Dassa and Bouige, 2001; Saier and Paulsen, 2001). The different ABC transporters have selectivity for various substrates, ranging from metabolic byproducts to physiologically significant signaling molecules such as peptides, lipids, cyclic nucleotides, and immunomodulators (see Table 1). A subset of these transporters (ABC1, or Pgp, ABCG2, several members of the ABCB sub-family, and possibly ABCA2) have been linked to multidrug resistance (MDR; Dean et al., 2001; Saier and Paulsen, 2001). MDR results from an increased capacity for efflux of both the original drug as well as other unrelated compounds via amplification, overexpression, or modification of these transporters.

By definition, ABC transporters contain at least one cytoplasmic ATP binding cassette, a highly-conserved ATPase domain containing a specific signature motif linked to WalkerA and WalkerB motifs that are characteristic of ATPases (Hyde et al., 1990). Some of the best-studied ABC transporters contain two ATP-binding cassettes alternating with two membrane-spanning domains (e.g., Pgp); these are considered full transporters, while those containing a single domain are classified as half transporters (Ambudkar et al., 2003; Sheps et al., 2004; Szakacs et al., 2006). The various ABC transporters cluster into eight families, designated ABCA through ABCH. Members of two of these families (ABCE and ABDF) contain two NBDs, but no transmembrane domains. Although they are not known to exhibit any transporter function, their NBDs appear to be derived from other ABC transporter classes, and they are therefore included with the transporters (Dean et al., 2001). These variations on ABC transporter domain structure are depicted in Fig. 1A. In humans, 48 ABC transporter genes representing seven of these families (ABCA to ABCG) have been identified, while representatives of all eight families can be found in the Drosophila and zebra fish genomes (Dean and Allikmets, 2001; Dean and Annilo, 2005; Annilo et al., 2006). Schistosome genomes code for approximately 20 transporters in 6 of these 8 sub-families; to date, no genes for ABCD or ABCH transporters have been detected (Greenberg, 2013a).

Examples of potential signaling molecules that are substrates of ABC transporters. Their possible functions, with an emphasis on those relevant to immunomodulation.

| Compound | Functions | Transporter(s) | References |
|----------|-----------|----------------|------------|
| Platelet activating factor (PAF) | Mediator of inflammation | ABCB1 (Pgp) | Ernest and Bello-Reuss (1999), Ragers et al. (2001) |
| Phospholipids [e.g., phosphatidylcholine, phosphatidylycerine (PS), phosphatidylethanolamine] | Membrane integrity, cell cycle regulation; cell signaling; schistosome PS and lyso-PS polarize DCS | ABCB1, ABCB3, ABCA1, ABCA2, ABCG2 (BCRP) | Pohl et al. (2002), Aye et al. (2009), Robb et al. (2003), Chen et al. (2008), Leier et al. (1994), Cui et al. (1999), Rius et al. (2008), Reid et al. (2003) |
| Cyclic nucleotides | Regulate inflammatory responses, monocyte polarization, maturation | ABCC4, ABC5, ABC11 | Wielinga et al. (2003), Chen et al. (2001), Guo et al. (2003) |
| Sphingosine-1-phosphate (S1P) | T-cell homing; immunosuppression | ABCB1 | Honig et al. (2003) |
| Leukotriene LTC4 | Mediator of inflammation; DC migration | ABCB1, ABCC4 | Leier et al. (1994), Cui et al. (1999), Rius et al. (2008), Reid et al. (2003) |
| LTB4, LTD4, LTE4 | Mediators of inflammation; DC migration | ABCB1, ABCC4 | Kim et al. (2008) |
| Prostaglandins PGE1, PGE2, PGE2a | Mediators of inflammation; DC migration/maturation; immune suppression | ABCB1, ABCC4 | Koopmann et al. (1996) |
| Sphingomyelin, glycolipids, cholesterol | Antigen presentation | C. elegans ABCA, ABCB6, ABCD, ABCG1 | Sundaram et al. (2006) |
| Peptides | TLR3 activation in DCs by schistosome eggs | | |
The critical association of ABC transporters with drug resistance and MDR has been a major driving force for much of the research on these proteins. As has been pointed out however, these transporters did not evolve to protect cancer cells and pathogens from medical interventions; instead, their ability to exclude toxins and xenobiotics from cells and tissues is likely co-opted and selected by drug treatment (Sarkadi et al., 2006). The most likely primary physiological role of these transporters is to act as a cellular defense system to protect cells and tissues from toxic agents. Interestingly, Sarkadi et al. (2006) have suggested that ABC transporters are part of a “chemoimmunity” network with innate and adaptive phases and features reminiscent of classical immunology. The adaptive phase is postulated to rely on a type of “memory” that depends on changes in transporter expression, efficiency, and coupling to other transporters and the general metabolic milieu, all of which are altered following an encounter with a particular toxin.

One manifestation of this protective function that has huge physiological implications occurs in vital tissues and organs requiring special protection (e.g., the brain), and is exemplified by the blood–brain barrier (BBB). ABC transporters such as Pgp and BCRP (ABCG2) serve as part of the mechanism in the BBB that blocks entry of potentially toxic compounds into the central nervous system (CNS) (Borst and Elferink, 2002). This barrier function of Pgp is highly relevant to at least one anthelmintic, the anti-nematodal drug ivermectin. The main target for ivermectin is the invertebrate-specific glutamate-gated chloride channel (Cleland, 1996; Wolstenholme, 2011); schistosomes also contain these channels, but they appear to be insensitive to ivermectin (Dufour et al., 2013). Part of the excellent safety profile of ivermectin also results from the fact that it is a substrate for mammalian Pgp, which mediates its exclusion from the host CNS. Loss or disruption of this Pgp function in the mammalian host can lead to ivermectin-induced neurological toxicity (Schinkel et al., 1994; Mealey et al., 2001). PZQ also has an outstanding safety profile, perhaps reflecting selective affinity for a platyhelminth-specific target (Greenberg, 2005b; Chan et al., 2012). However, there have been sporadic reports that PZQ can also affect molluscan and mammalian cells (Chubb et al., 1978; Gardner and Brezden, 1984). We have speculated (Greenberg, 2013a) that perhaps PZQ exclusion from the CNS (via mechanisms similar to those seen for ivermectin) is responsible in part for the excellent safety profile of the drug. On the other hand, PZQ has been reported not to be a substrate for mammalian uptake of xenobiotics (typically phosphatidylerine and phosphatidylethanolamine) against a concentration gradient, towards the cytoplasmic face of the membrane. Floppases, exemplified by ABC transporters, translocate substrates (e.g., phosphatidylcholine, sphingolipids, cholesterol) in the opposite direction. Scramblases (not shown) are ATP-independent and calcium-dependent and transport lipids in both directions, along the concentration gradient, disrupting membrane asymmetry (Daleke, 2007; Sharom, 2011a).

4. Physiological functions of ABC transporters

Based on several lines of evidence, including the crystal structures of both mammalian (Aller et al., 2009; Li et al., 2014) and Caenorhabditis elegans (Jin et al., 2012) Pgp (Fig. 1B), the "hydrophobic vacuum cleaner" model is thought by most researchers to be a primary mechanism by which compounds interact with the binding pocket of Pgp. This model posits that Pgp “captures” these substrates while they are within the inner leaflet of the lipid bilayer (although access from the cytoplasm is also possible). Upon binding of ATP, Pgp either pumps or flips these substrates to the outer leaflet of the membrane or to the extracellular medium (Higgins and Gottesman, 1992; Sharom, 2011b). Jin et al. (2012) recently provided evidence in support of this model, finding that Pgp substrates within the membrane had up to a 4000-fold higher affinity for Pgp than those in detergent.
Pgp (though it is an inhibitor; Hayeshi et al., 2006); one might speculate that a different ABC transporter such as BCRP is used to exclude PZQ from the host CNS. We are, however, unaware of any studies that test whether PZQ can act as a substrate for mammalian BCRP.

Several lines of evidence suggest that ABC transporters also play critical roles in normal cellular and organismal biology beyond this predominant protective activity (reviewed by Johnstone et al., 2000a; Mizutani et al., 2008). These include, among others: regulation of cell differentiation, proliferation, and survival, including apoptosis (Johnstone et al., 2000b); tumor promotion (Fletcher et al., 2010); and modulation of immune function (Mizutani et al., 2008; van de Ven et al., 2009a,b; Seyffir and Tampe, 2014). Interestingly, PZQ has been shown to synergistically enhance the efficacy of paclitaxel, a Pgp substrate, in inhibiting cancer cell growth, markedly decreasing expression of an anti-apoptotic protein (Wu et al., 2012). Additionally, mutations in ABCA transporters associated with several disease states and members of this subfamily have been implicated in neurodegenerative disorders such as Alzheimer’s Disease (reviewed in van Meer et al., 2006; Kim et al., 2008; Piehl et al., 2012). Furthermore, as summarized in Table 1 and discussed above, many known or potential signaling molecules can serve as high-affinity substrates of different ABC transporters (Borst et al., 2000; Pohl et al., 2002; Sundaram et al., 2006; Mizutani et al., 2008; Aye et al., 2009; Quazi and Molday, 2011). As has been pointed out however (Borst et al., 1998; Borst and Elferink, 2002), some caution is in order when interpreting activities found under artificial experimental conditions (e.g., transporter inhibitors with variable selectivity and potency, gross overexpression of transporter proteins) as being reflective of genuine functions within the organism. Newer molecular genetic approaches may ultimately resolve some of this ambiguity.

5. ABC transporters in schistosomes

5.1. Drug susceptibility

We and others have recently reviewed the evidence that ABC transporters can influence drug susceptibility and may be associated with development and maintenance of drug resistance in schistosomes and other parasitic helminths (Kerboeuf et al., 2003; James et al., 2009a; Kasinathan and Greenberg, 2012; Lespine et al., 2012; Ardelli, 2013; Greenberg, 2013a). The association between helminth ABC transporters (most typically Pgp) and levels of anthelmintic susceptibility (and insusceptibility) is becoming more apparent, though a clear-cut, direct causal link between changes in transporter structure or expression and drug resistance remains elusive. However, we have shown that PZQ is an inhibitor and likely substrate of recombinant S. mansoni Pgp (Kasinathan et al., 2010), suggesting a mechanism by which disruption of Pgp function might increase effective PZQ concentration. Indeed, preliminary evidence from our group indicates that disruption of S. mansoni ABC transporter expression or function can potentiate the antischistosomal effects of PZQ (Greenberg et al., 2013). Pgp also appears to be associated with triacendazole (TCBZ) resistance in another nematode, the liver fluke Fasciola hepatica (Wilkinson et al., 2012), and the Pgp inhibitor dexverapamil can enhance TCBZ activity in vitro against a TCBZ-resistant F. hepatica isolate (Savage et al., 2013). Similarly, inhibition of Pgp can enhance sensitivity of the nematode C. elegans to ivermectin (Ardelli and Prichard, 2013). These types of studies could potentially lead to new therapeutic strategies that combine anthelmintics with currently-approved drugs that, in addition to acting on their primary targets, block Pgp or other ABC transporters. If successful, such strategies could improve drug efficacy and possibly prevent emergence of drug resistance.

5.2. Excretory activity

Initial inquiries into genuine physiological functions of ABC transporters in schistosomes began over a decade ago, using fluorescent substrates of Pgp and MRP to visualize the parasite excretory system. The putative Pgp substrate resorufin exhibited energy-dependent concentration of fluorescence in the excretory tubules of adult worms (but not schistosomules), and allowed visualization of excretory activity via the nephridopore (Sato et al., 2002). Pgp inhibitors disrupted this excretory activity. Subsequent experiments using fluorescent MRP substrates showed similar results (Sato et al., 2004). Notably, PZQ (as well as other agents) disrupts the localization of resorufin, most potently in males (Kusel et al., 2006, 2009). Remarkably, an experimentally-induced PZQ-resistant isolate of schistosomes (LE-PZQ) is refractory to these effects of PZQ on resorufin labeling of the excretory system (Couto et al., 2010). As has been pointed out (Kusel et al., 2009), the excretory system of schistosomes is essential to the parasite’s survival, and ABC transporters that function within it offer candidate targets for disruption of vital parasite functions such as metabolic regulation, excretion of drugs and other toxic compounds, and interaction with the host.

5.3. Reproduction

As discussed in a recent review (Greenberg, 2013a), a 2003 patent showed that the calcium channel blockers verapamil and nifedipine significantly reduced egg production in both S. mansoni and the intestinal fluke Echinostoma caproni (Walter and Kuris, 2003). In addition to their activity against calcium channels, however, both of these drugs are relatively potent inhibitors of mammalian ABC transporters (Kasinathan et al., 2010). Using a combination of molecular genetic and pharmacological approaches, we confirmed the reported results on egg production in S. mansoni, and showed that these effects were attributable to interference with schistosome ABC transporters (Kasinathan et al., 2011). Furthermore, within the infected mouse host, Pgp and MRP1 inhibitors significantly reduced liver egg burden and pathology (Kasinathan et al., 2011).

The role ABC transporters might be playing in schistosome egg production is not yet clear. Eggs produced by worms exposed to ABC transporter inhibitors were often morphologically abnormal. However, isolated mature eggs exposed to these drugs appeared to be viable and hatched normally, suggesting that the transporter inhibitors are affecting egg development rather than the mature eggs directly. Limited analysis of the schistosome reproductive system using confocal microscopy suggested that the defect mapped primarily to females, and appeared to be manifested as a loss of immature oocytes and “piling up” of mature oocytes. Consistent with a female-specific effect, females exposed to transporter inhibitors could not be rescued by untreated males; they continued to exhibit reduced egg production (Kasinathan et al., 2011). Nonetheless, we have observed some limited evidence for a role of ABC transporters in male reproductive development as well. Clearly, a more rigorous and thorough examination of the role of ABC transporters in schistosome egg production is warranted, and could lead to new insights into development of the parasite reproductive system. It will also be important to determine whether these effects on egg production reflect a direct role for ABC transporters in this function or are rather a “collateral damage” due to interference with the activity of other organ systems dependent on these transporters, such as the gut or the excretory system (interestingly, such a situation may be occurring with ivermectin, which, as discussed below, interferes with excretory activity of filarial worms and suppresses production of microfilariae). Regardless of mechanism, however, as we have pointed out (Kasinathan et al., 2011;
Greenberg, 2013a), these results could have practical implications for disease treatment and control strategies. Schistosome eggs cause the majority of pathology in schistosomiasis and they are also the agents of disease transmission. Use of agents that decrease parasite egg production, alone or in combination with anthelmintics, could be important in limiting host pathology and spread of drug resistance.

5.4. Parasite–host interactions

Several studies have implicated mammalian ABC transporters in influencing immune function (Mizutani et al., 2008; van de Ven et al., 2008, 2009a). ABC transporters have been linked to immunological functions such as Th1 skewing and activation (Pendse et al., 2006; Kooij et al., 2009), T cell migration (Randolph et al., 1998; Honig et al., 2003), and DC maturation and migration (Randolph et al., 1998; van de Ven et al., 2008; Kooij et al., 2009; van de Ven et al., 2009b). As discussed above, ABC transporters can translocate a wide array of compounds with biological signaling capability (Table 1), and in some cases, transporters and their substrates have been linked directly to functional outputs. TAP1 and TAP2, heterodimeric ABC transporters (ABC2B + ABC3), deliver cytosolic peptides to class I MHC molecules for antigen presentation (Koopmann et al., 1996; McCluskey et al., 2004; Hinz and Tampe, 2012; Seyffir and Tampe, 2014). ABC transporters have also been implicated in pro-inflammatory cytokine efflux (Drach et al., 1996; Raghu et al., 1996; Frank et al., 2001; Pavlik et al., 2005; Pendse et al., 2006), though mice lacking mdr1a-encoded Pgp exhibit relatively normal cytokine levels and T-cell function (Eisenbraun and Miller, 1999). Others have suggested that ABC transporters may influence cytokine production indirectly, through bioactive lipid compounds that are substrates of ABC transporters (Kooij et al., 2012). There is also evidence that cytokines themselves can influence ABC transporter expression in immune cells (Liptrott et al., 2009).

Schistosomes and other parasites are hugely successful at, and indeed depend for their survival and development on, modulating and manipulating host immune responses (Pearce and Macdonald, 2002; Lamb et al., 2010). How the host immune system responds to the parasite determines in large part the balance between protective immunity (health) and immunopathology (morbidity) (Wilson et al., 2007). Interestingly, others have suggested that immune interference with the microflarial excretory-secretory apparatus in filarial worms might be altering parasite modulation of host responses, contributing to the rapid clearance of microfilariae from the host (Moreno et al., 2010). Could schistosomes and other parasites use their ABC transporters as part of the apparatus by which they influence host responses?

Substrates of ABC transporters with potential immune signaling activity (see Table 1) include compounds implicated in schistosome modulation of host responses such as glycolipids (Van der Kleij et al., 2002b), lipids and phospholipids (e.g., phosphatidylserine and lyso-phosphatidylserine; van der Kleij et al., 2002a; van Riet et al., 2009), and dsRNAs (Aksoy et al., 2005). Excretory/secretory products of schistosomules and adults contain classes of immunomodulators such as eicosanoids (Safalysky and Fusco, 1987; Angeli et al., 2001) that can serve as high-affinity ABC transporter substrates. Furthermore, schistosomule-derived prostaglandin D2 inhibits migration of epidermal Langerhans cells, dendritic cells that play a key role in establishing cutaneous immunity, to the skin-draining lymph nodes (Angeli et al., 2001). Interestingly, in our studies on the role of schistosome ABC transporters in egg production, we found that S. mansoni-infected mice administered Pgp inhibitors showed not just a reduction in liver granuloma number (corresponding to the lower egg burden), but also a significant decrease in granuloma size (Kasinathan et al., 2011).

Such a difference in granuloma size is not predicted or explained by the reduction in egg burden, and could indicate an altered host immune response to the parasites, perhaps reflecting an effect of the Pgp inhibitors on the immunomodulatory properties of the eggs (or, of course, on host cells, or on both).

These observations suggest that schistosome (or other helminth) ABC transporters may have been co-opted by parasites to constitute part of the mechanism used to shape and manipulate host responses. Clearly, this question is worth pursuing, as it could provide information on novel roles for this class of transporters as well as possible new therapies that interfere with this function. By framing the questions correctly, the combination of the rich pharmacology of these transporters with the availability of powerful molecular genetic approaches should allow researchers to dissect the role of ABC transporters in these complex interactions, and could provide important insights into the mechanisms underlying parasite–host interactions.

6. Conclusions

This review has outlined two parallel inquiries regarding the role of ABC transporters in schistosomes. First, there is increasing evidence that schistosome (and other helminth) ABC transporters can modulate PZQ sensitivity. If that is indeed the case, it may provide the impetus to test repurposed ABC transporter inhibitors in combination therapy to potentiate PZQ activity. This prospect is exciting, as it could increase the real-world effectiveness of PZQ, particularly against immature worms, as well as prevent emergence or spread of resistance. As we have discussed previously (Kasinathan and Greenberg, 2012; Greenberg, 2013a), such an approach would likely involve only one or two doses in combination with standard PZQ treatment, thereby reducing the likelihood of encountering the types of complications seen with long-term, high-dose regimens tested in clinical trials to enhance cancer chemotherapy (S zakacs et al., 2006; Shukla et al., 2008; Coley, 2010).

The second line of inquiry focuses on the genuine functions of these transporters in the parasite. Though current work has barely scratched the surface of this topic, it seems obvious that ABC transporters are likely to be quite important for schistosome survival and development within its two very different hosts, if only to keep toxins and xenobiotics in check. Given the limited number of studies focused on these proteins in parasites, it is quite striking that they have already been implicated in schistosome excretory activity and reproduction, two functions that are vital to parasite survival, transmission, and pathogenicity. The possibility that schistosome ABC transporters are involved in presentation of parasite immunomodulatory factors to the host is intriguing, and if validated, suggests yet another novel function for members of this ancient and highly diverse protein superfamily.

Conflict of interest

The author declared that there is no conflict of interest.

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