Mechanisms of molluscan host resistance and of parasite strategies for survival

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SUMMARY

In parallel with massive research efforts in human schistosomiasis over the past 30 years, persistent efforts have been made to understand the basis for compatibility and incompatibility in molluscan schistosomiasis. Snail plasma contains molecules that are toxic to trematodes, but these seem to kill only species that never parasitize the mollusc used as the source of plasma. A sporocyst will be killed actively by haemocytes alone if they are from a snail that is resistant to the trematode. Oxygen-dependent killing mechanisms play a major role. Enzymes such as NADPH oxidase, superoxide dismutase, myeloperoxidase and nitric oxide synthase are critical components of the putative killing pathways. Metabolic intermediates such as hydrogen peroxide and nitric oxide appear to be more important against trematodes than the shorter-lived intermediates that are more important in anti-microbial defences. Products secreted by trematode larvae influence the physiology of snail haemocytes, implying active counter-defences mounted by the parasite, but these remain largely unexplored. A possible molecular basis for the susceptibility/resistance dichotomy in molluscan schistosomiasis is suggested to be deficient forms of enzymes in the respiratory burst pathway, and a selective disadvantage for schistosome resistance is an integral component of this model.

Key words: *Schistosoma*, *Biomphalaria*, compatibility, susceptibility, resistance, oxygen-dependent killing, haemocyte, cytotoxicity, myeloperoxidase, hydrogen peroxide, nitric oxide.

INTRODUCTION

What an exciting time it is to be re-examining aspects of the relationships between flukes and snails. In the thirty years since Wright's completion of his book (*Flukes and Snails*, Wright, 1971 *a*), a great deal has been learned about trematodes and their intermediate hosts. Yet, with local exceptions, progress in reducing the numbers of infected people and livestock has not been impressive. New anti-trematode drugs have become available, but remain unaffordable to affected populations and do not provide protection from re-infection. For many individuals in endemic areas, vaccine development remains the leading hope; and that (hope) is the key word.

Many of those who contributed to this volume surely owe debts of gratitude to Wright. One of us (C. J. B.) began work in this field in 1971, and one's perspective on things then was constructively influenced by Wright's views on, for example, 'Susceptibility–Infectivity–Compatibility'.

Notwithstanding the success of Wright's book in provoking constructive and productive mind-sets and encouraging further research, the limits of knowledge at the time forced him to be satisfied to pose probing questions and suggest likely answers. At a time when the information was simply not there to proceed otherwise, Wright's way of dealing with immunity, for example, was to posit realistic scenarios, such as paraphrased here: 'That the evasion of the innate host response is an active process on the part of the parasites is emphasized by the fact that immediately after they die they are attacked and encapsulated prior to removal.'

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HOST RESISTANCE

The heading above really is an oxymoron and makes sense only in the context of the kind of host–parasite relationships exemplified by snails and trematodes. In general, when a trematode enters the body of a mollusc, it enters a hostile world. Unless the genetic profiles of the mollusc and the trematode are precisely concordant in some critical attributes, the parasite is killed. Thus one thinks of *individuals* in a population of the host species as being susceptible or resistant to *individuals* of the parasite species (Basch, 1975). Death, which awaits miracidia/sporocysts entering any non-susceptible individual, may be mediated by either humoral or cell components of the snail, or both. There is, indeed, a multiplicity of factors that contribute to the outcome of each host-parasite encounter, and this is well illustrated by the notion of encounter filters and compatibility filters, eloquently developed by Combes (1991).

Humoral and cellular components of host defences

It is likely that plasma molecules comprise one type of barrier. However, this may be operative only in cases of snail species that are resistant to all individuals of a given trematode species. This, of

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course, includes the majority of potential host-parasite pairs. This conjecture is based on recent data (Sapp & Loker, 2000*a*) implicating snail plasma factors as the agents of rapid (minutes to a few hours) killing of trematodes *in vitro*. Such toxic effects of plasma appear limited to trematodes that penetrate snails that are only distantly related to their normal host species. Thus flukes that parasitize planorbids are killed in plasma from lymnaeid snails, and *vice versa*. Earlier work (Bayne, Buckley & DeWan, 1980; Granath & Yoshino, 1984) had suggested that the presence of plasma from resistant planorbids enables susceptible haemocytes to kill *Schistosoma mansoni* sporocysts *in vitro*. However, this was not a potent effect and required long culture times. It later became apparent that the extra-cellular haemoglobin in *Biomphalaria glabrata* plasma will become oxidized and consequently generate hydrogen peroxide when exposed to normoxic conditions (Bender *et al*. 2001). Such events probably account for some, though perhaps not all, of the toxicity observed in the earlier studies.

While plasma toxicity appears not to be a problem for flukes entering individuals of their host or closely related species (Sapp & Loker, 2000*a*), such snails are fully immunocompetent and deal normally with other immune challenges. Susceptibility is specific to a narrow parasite genotype. How, then, is survival achieved when a trematode is confronted with the impressive internal defensive armoury of an individual compatible snail (Bayne & Yoshino, 1989; van der Knaap & Loker, 1990; Loker, 1994)? Recent studies (Hahn, Bender & Bayne, 2001*a*, *b*) have made it possible to construct an appealing model of how compatible and incompatible phenotypes may come about (see below), and they have potentially brought us to the brink of grasping the subtleties of at least some successful trematode–snail parasitisms.

The dominant effectors of sporocyst killing in resistant individuals of a host species are the haemocytes. In general, pulmonates seem to have two types of haemocyte, granulocytes and hyalinocytes. Definitive experiments have not been done to exclude the possibility of a cytotoxic role for the hyalinocytes, but ultrastructural evidence garnered in several laboratories implicates the granulocytes as the effector cells. When sporocysts and haemolymph are allowed to interact *in vivo* or *in vitro*, cells contacting the parasite appear to be granulocytes (Bayne *et al*. 1980; Loker *et al*. 1982). These data are consistent with those obtained in different host-parasite associations. Even when sporocysts of *S. mansoni* come into contact *in vitro* with haemocytes from resistant strains of *B. glabrata* in the absence of snail plasma (it is replaced with a culture medium), the parasites are killed. This killing is effected as fast as in whole haemolymph, and (unpublished observations) requires only sufficient cells to adhere to less than half of the sporocyst's surface. As complete envelopment of the parasite in haemocyte encapsulations is not needed for a kill, it is clear that killing is an active process and not due simply to suffocation or starvation.

Does this leave any room for a plasma role in parasite killing in host–parasite pairs in which some individuals are compatible? Evidence from *in vivo* experiments is equivocal. Injections of plasma from resistant strains were reported to decrease infection frequencies in normally susceptible individual *B. glabrata* (Granath & Yoshino, 1984). Furthermore, when susceptible individuals were implanted with haematopoietic tissue from resistant individuals, in which the implants survived for weeks, the implanted individuals were less susceptible to miracidial challenge (Sullivan & Spence, 1994; 1999). In such snails, implanted cells may be able to synthesize and secrete plasma components, but the implanted tissue almost certainly gives rise to haemocytes with the resistant phenotype. The favoured interpretation is that resistance has been adoptively transferred and is mediated by the new cells.

Regardless, in completely plasma-free conditions, haemocytes of the 13-16-R1 strain of *B. glabrata* kill sporocysts of the PR1 strain of *S. mansoni* without reduced efficacy (Hahn *et al*. 2001*a*).

The defensive armoury of the molluscan haemocyte

In the 1950s, 1960s and 1970s, both light and electron microscopy had been used to learn much about the organelles within molluscan haemocytes, and to observe the structures of cells responding to parasites and other foreign agents (the work of M. R. Tripp, T. C. Cheng and others, reviewed by Harris, 1975, and Bayne, 1983). Most of what was learned was, in retrospect, unsurprising. Haemocytes that circulate as free cells *in vivo* are typical phagocytic leukocytes: they will aggregate in response to trauma, phagocytose small foreign particles (bacteria, yeast), and encapsulate large ones (trematode larvae). By means of cytochemical stains, it was found that these cells contain lysosomes and these fuse with phagosomes releasing their digestive enzymes into the phagolysosomal vacuole whose membrane's proton pumps ensure a transient decrease in pH (Kroschinski & Renwrantz, 1988). Into the plasma, haemocytes may release enzymes. In a reciprocal manner, haemocyte behaviours are facilitated by constitutive plasma molecules, such as opsonizing lectins that bind to yeast thus targeting them for phagocytosis (Fryer, Hull & Bayne, 1989). Evidence of chemotactic behaviour proved elusive, but was eventually found (Kumazawa & Shimoji, 1991).

A little of what was learned in those early studies was perplexing and remains unexplained. For

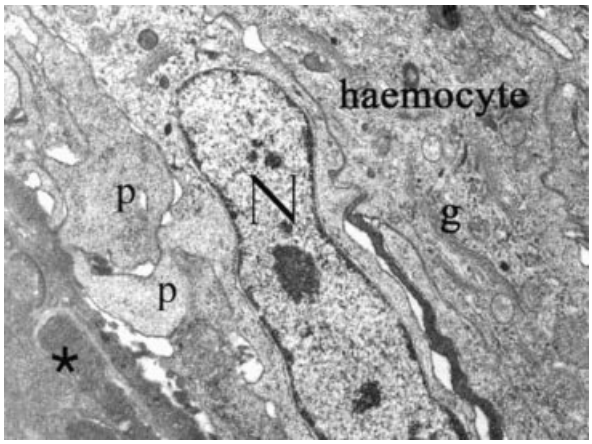

Fig. 1. Encapsulated and killed *Schistosoma mansoni* sporocyst (dark region of image, bottom left, *) within a group of haemocytes from the 10-R2 strain of *Biomphalaria glabrata*. The encapsulation occurred in a juvenile snail, and was fixed 24 h after it was exposed to miracidia. The cytoplasm of the haemocyte to the right of the nucleus (N) contains numerous organelles, including a Golgi apparatus (g). Close to the sporocyst surface, two pseudopods (p) display a finely granular cytoplasm that is free of organelles. (E. S. Loker & C. J. Bayne, unpublished.)

example, a glycocalyx on the haemocyte surface was found to be redistributed to the 'far' side of the *Bulinus guernei* haemocyte when the cells came close to a *S. haematobium* sporocyst (Krupa, Lewis & Del Vecchio, 1977). Intracellularly, the cytoplasm of *B. glabrata* haemocytes in contact with *S. mansoni* sporocysts was found to be finely granular and organelles were absent (Fig. 1; see also Loker *et al.* 1982). The meaning of these structural observations remains unexplored. Among remaining mysteries is this: *in vivo* encapsulations, naturally, grow to an effective size that is appropriate to the task; but the nature of the graded signal that terminates further cell accumulation remains unknown. And in multilayered capsules, any differences in the functional roles of cells in actual contact with the target and those without contact remain unclear.

Building on earlier reports of the existence of lectins that serve as cell surface receptors (pattern recognition molecules) for carbohydrate structures (pathogen-associated molecular patterns, Janeway, 1989), evidence has recently been obtained for physiological functions of surface lectins on snail haemocytes (Hahn, Bender & Bayne, 2000). Development of this evidence exploited the respiratory burst potential of snail haemocytes (see below). Eight sugars, six of which are known to occur on the *S. mansoni* sporocyst surface, were tested for their ability to stimulate this response. No free sugars elicited the respiratory burst. However, *B. glabrata* haemocytes responded by producing reactive oxygen species when stimulated with BSA-galactose, BSA-mannose and BSA-fucose. In contrast, BSA conjugated with *N*-acetyl glucosamine, *N*-acetyl-galactosamine, lactose, glucose or melibiose was without effect. The responses were shown to be dependent on NADPH-oxidase. Since they were similar in cells from a susceptible and a resistant strain, it appears that the cells did not differ with respect to their lectin-type surface receptors. These results (1) imply that haemocytes from both strains are equally capable of producing reactive oxygen species, (2) imply that receptor activation requires a patterned presentation of multiple ligands, and (3) suggest that the susceptible-resistant dichotomy is likely due to something other than different recognition capabilities, though the possibility of strain-specific discrimination of more complex glycan structures has not been excluded.

The more molecular work that has typified recent research has revealed evidence for antimicrobial peptides in molluscan haemocytes (Charlet *et al.* 1996; Mitta, vandenBulckle & Roch, 2000; Mitta *et al.* 2000), though no evidence has been reported for toxicity of these peptides to metazoan parasites.

THE HOST–PARASITE MODEL

We have taken advantage of strains of *B. glabrata* that were developed by C. S. Richards (Richards & Merritt, 1972), and of the PR1 strain of *S. mansoni*. Both the snail and the trematode have been maintained in the laboratory for decades. Such models have attracted the criticism that what occurs in them may no longer represent events in the real world. Even though these inbred strains may not represent the full array of natural scenarios, they clearly lead to normally infective parasites: cercariae shed from susceptible individuals are fully infective for mammalian hosts. Furthermore, findings obtained through studies on inbred laboratory strains serve to do no more than direct ideas as to what factors may operate in the real world.

What has been repeatedly confirmed by subsequent research is that the haemocytes from individual snails that are resistant to PR1 *S. mansoni* are effective sporocyst killers on their own (Bayne *et al.* 1980; Hahn *et al.* 2001*a*). A potential mechanism of this cell-mediated killing became apparent in studies involving the production of reactive oxygen species (ROS) by haemocytes. Dikkeboom and coworkers first demonstrated that gastropod haemocytes produce ROS (Dikkeboom *et al.* 1988*a*), and that they do so in response to encounters with trematodes (Dikkeboom *et al.* 1988*b*). Simultaneously, Shozawa, Suto & Kumada (1989) reported that *B. glabrata* haemocytes produce ROS. A direct involvement of ROS in haemocyte-mediated killing of trematodes was first demonstrated by Adema *et al.* (1994*b*), using sporocysts of the avian schistosome *Trichobilharzia ocellata* and haemocytes

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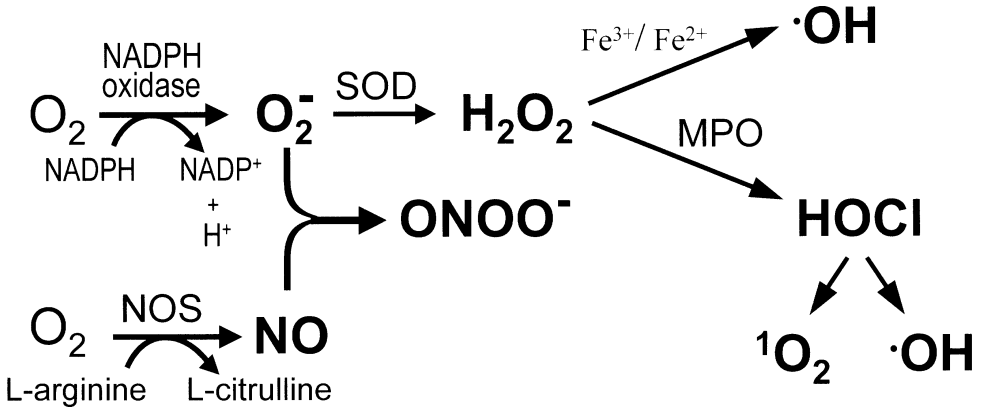

Fig. 2. Simplified schematic of the pathways that generate the major reactive oxygen and nitrogen species in phagocytic cells. The membrane-bound NADPH oxidase complex converts molecular oxygen (O_2) into superoxide (O_2^-), which can spontaneously dismutate to hydrogen peroxide (H_2O_2). However, this process is greatly accelerated by the enzyme superoxide dismutase (SOD). In the presence of chloride ion, H_2O_2 can be converted to hypochlorous acid (HOCl) by the enzyme myeloperoxidase (MPO). H_2O_2 can also react with iron (Fe^{3+}) to yield hydroxyl radical (·OH; Fenton reaction). HOCl can potentially produce both ·OH and singlet oxygen ($^1O_2$). The enzyme nitric oxide synthase (NOS) utilizes O_2 and arginine to produce nitric oxide (NO), which can react with O_2^- to form the highly reactive peroxynitrite ($ONOO^-$).

from its intermediate host, *Lymnaea stagnalis*, as well as *S. mansoni* and *B. glabrata*. This study showed that when the generation of ROS by haemocytes was prevented, the haemocytes' ability to kill the parasite was compromised. Together, these studies paved the way for a more detailed examination of the contribution of ROS to parasite killing.

The burst of oxygen consumption (i.e. respiratory burst) by phagocytic cells and the consequent production of ROS is the result of activation and assembly of an NADPH oxidase complex in the phagocyte plasma membrane (reviewed by Babior, 1999). This enzyme complex catalyzes the production of superoxide (O_2^-), which serves as the first radical in the chain of production of additional reactive oxidants: hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), hypochlorous acid (HOCl), and singlet oxygen ($^1O_2$) (Fig. 2; reviewed by Hampton, Kettle & Winterbourn, 1998). Additionally, most phagocytic cell types possess an inducible nitric oxide synthase (iNOS), which generates nitric oxide (NO) from molecular oxygen and arginine. Although NO has cytotoxic activity on its own, it rapidly reacts with O_2^- to form the much more reactive oxidant, peroxynitrite ($ONOO^-$). In mammalian neutrophils, most of the O_2^- produced is converted to H_2O_2 (Makino *et al*. 1986) and most of the H_2O_2 is consumed by myeloperoxidase (MPO), an enzyme that uses H_2O_2 and chloride ion to produce HOCl, the most potent antibiotic produced by defence cells. Like their mammalian counterparts, molluscan haemocytes generate HOCl (Schlenk, Martinez & Livingstone, 1991; Torreilles, Guerin & Roch, 1997) and NO (Conte & Ottaviani *et al*. 1995; Torreilles & Guerin, 1999; Arumugam *et al*. 2000). Thus, haemocytes have the potential to produce both highly reactive (O_2^-, ·OH, $^1O_2$, HOCl and $ONOO^-$) and relatively long-lived (NO and H_2O_2) oxidants. Each oxidant differs in its reactive properties (e.g. ability to cause DNA strand breaks, lipid peroxidation, enzyme inactivation, etc.). The precise roles of particular oxidants in pathogen killing remain unclear. Studies with MPO-deficient mice indicate the relative cytotoxic roles of specific oxidants are dependent on the bacteria or fungi involved (Aratani *et al*. 2000). Thus, it seems likely that pathogens employ a variety of anti-oxidant strategies and effective killing requires the production of the right oxidant at the right time and place. In light of these facts, we sought to determine the specific reactive oxidant pathways that allow resistant *B. glabrata* haemocytes to kill sporocysts of *S. mansoni*.

Utilizing an *in vitro* assay, killing of *S. mansoni* (PR 1) sporocysts by haemocytes from resistant (13-16-R1) *B. glabrata* was examined over a 48 h time-course. To determine which ROS is involved, specific oxidant scavengers or enzyme inhibitors were used (Hahn *et al*. 2001*a*, *b*). Addition of superoxide dismutase (SOD), which scavenges O_2^-, and addition of hypotaurine, which scavenges hypochlorous acid (HOCl) and hydroxyl radicals (·OH), did not protect sporocysts from being killed. In addition to eliminating HOCl and ·OH as direct mediators of sporocyst killing, these results exclude a cytotoxic role for singlet oxygen ($^1O_2$), since it is a product of HOCl. Addition of catalase, which converts H_2O_2 to water and molecular oxygen, did protect sporocysts significantly, indicating that H_2O_2 is the reactive oxygen species mainly responsible for sporocyst killing. These results are in contrast with results from mammalian studies which show that most of the H_2O_2 generated by neutrophils is consumed by the rapidly acting MPO and converted into HOCl (Kettle & Winterbourn, 1997). Although

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HOCl is considered to be a more potent bactericidal agent than H_2O_2 (Klebanoff, 1968), *S. mansoni* sporocysts are less susceptible to HOCl than to H_2O_2: in an *in vitro* toxicity assay, 300 μM HOCl was required to achieve 100% sporocyst mortality compared to 150 μM H_2O_2 (Hahn *et al*. 2001*a*). The possible involvement of NO in haemocyte-mediated killing of sporocysts was also examined. Addition of N_ω-nitro-L-arginine methylester (L-NAME), which inhibits both inducible and constitutive NOS activity, significantly reduced sporocyst killing. However, the presence of uric acid, which selectively scavenges $ONOO^-$, but does not react with NO, did not affect sporocyst killing.

In summary, these results indicate that H_2O_2 and NO are directly involved in haemocyte-mediated killing of *S. mansoni* sporocysts. Furthermore, these findings also suggest that HOCl, O_2^-, $^1O_2$, and $ONOO^-$ do not play a direct role in haemocyte-mediated killing. Since H_2O_2 and NO could be considered intermediates of the more toxic oxidants, HOCl, $\cdot OH$ and $ONOO^-$, these results came as somewhat of a surprise. It is possible that the highly reactive nature of HOCl, $\cdot OH$ and $ONOO^-$ limits their activity to the sporocyst tegument, which may be able to sustain substantial damage and recover due to its rapid turnover or its repair mechanisms. The less reactive nature of H_2O_2 and NO, coupled with their ability to cross membranes, may allow them to penetrate the tegument and act on targets within the sporocyst.

MECHANISTIC MODEL OF RESISTANCE/SUSCEPTIBILITY

The knowledge gained from these mechanistic studies provides the means to formulate hypotheses that address specific biochemical differences that may determine resistance/susceptibility. Since the biochemical pathways that produce H_2O_2 and NO appear to be directly involved in sporocyst killing, several hypotheses suggest themselves. Each addresses the possibility that susceptibility/resistance is mediated by a difference in one of these pathways.

Fig. 3 (A–C) illustrates potential 'enzyme deficiencies' in *B. glabrata* haemocytes that could promote susceptibility to *S. mansoni*. Since the production of H_2O_2 by haemocytes is essentially determined by the generation of O_2^- and its subsequent dismutation to H_2O_2, any anomalies involving either the NADPH oxidase complex or superoxide dismutase could potentially result in a decreased killing capacity (i.e. susceptibility). Similarly, anomalies involving NOS expression or activity could also lessen haemocyte-mediated killing. Regarding the first two scenarios, the evidence suggests that resistant and susceptible haemocytes do not differ in H_2O_2 production when stimulated with zymosan (Dikkeboom *et al*. 1988*a*) or with glycosylated albumin (Hahn *et al*. 2000). Attempts to compare H_2O_2 production by resistant and susceptible haemocytes after stimulation by live sporocysts have been inconclusive, due to high background as a result of sporocyst products interacting with the fluorescent probe. Although the possibility of different responses of resistant and susceptible haemocytes to *S. mansoni* sporocysts cannot be eliminated, all available evidence suggests that, in terms of H_2O_2 generation, the haemocytes from both strains are equally competent. The same case cannot be made for NO generation (Fig. 3C). It remains to be determined if resistant and susceptible haemocytes differ in their ability to produce NO.

Fig. 3D illustrates a potential enzyme deficiency in *B. glabrata* haemocytes that could promote resistance to *S. mansoni*. Two of our findings provide the basis for this scenario: haemocytes utilize H_2O_2 (and not HOCl) to kill sporocysts, and HOCl is significantly less toxic to *S. mansoni* sporocysts than H_2O_2. Thus, in terms of toxicity to *S. mansoni* sporocysts, haemocyte MPO would essentially function as a detoxifying enzyme. This presents the possibility that MPO activity prevents sporocyst killing in susceptible haemocytes by lowering the net quantity of H_2O_2 available for cytotoxic activity. A deficiency in this enzyme would allow accumulation of higher levels of H_2O_2, and thus promote resistance. This scenario is intriguing for two reasons. In human populations, MPO deficiency is the most common congenital neutrophil defect (Nauseef, 1990), with an estimated incidence of 1 in 2000–4000 in the United States (Parry *et al*. 1981). Even more interesting is the clinical significance of MPO deficiency. Most individuals with MPO deficiency are healthy and do not suffer from recurrent bacterial infections. However, increased susceptibility to fungal infections has been reported (Parry *et al*. 1981), and studies with MPO-deficient mice support the notion that the role of MPO in oxidative killing varies from critical to inconsequential depending on the pathogen (Aratani *et al*. 1999, 2000). Thus, in our hypothetical case of MPO-deficient snails, an increased ability to kill *S. mansoni* may come at the cost of an increased susceptibility to other pathogens (particularly fungus). The idea of 'resistance at a cost' in regard to schistosomes is not new. It stems from the perplexing observations that the prevalence of schistosome-susceptible snails is often quite high in both natural and laboratory populations of *Biomphalaria* sp. (Upatham, 1972; Fransden, 1979; Minchella & LoVerde, 1983). Wright suggested that resistant snails are not predominant because resistance is associated with a disadvantageous character or physiological defect (Wright, 1971*b*). We concur with Wright and postulate that this defect comes in the form of MPO deficiency; the dis-

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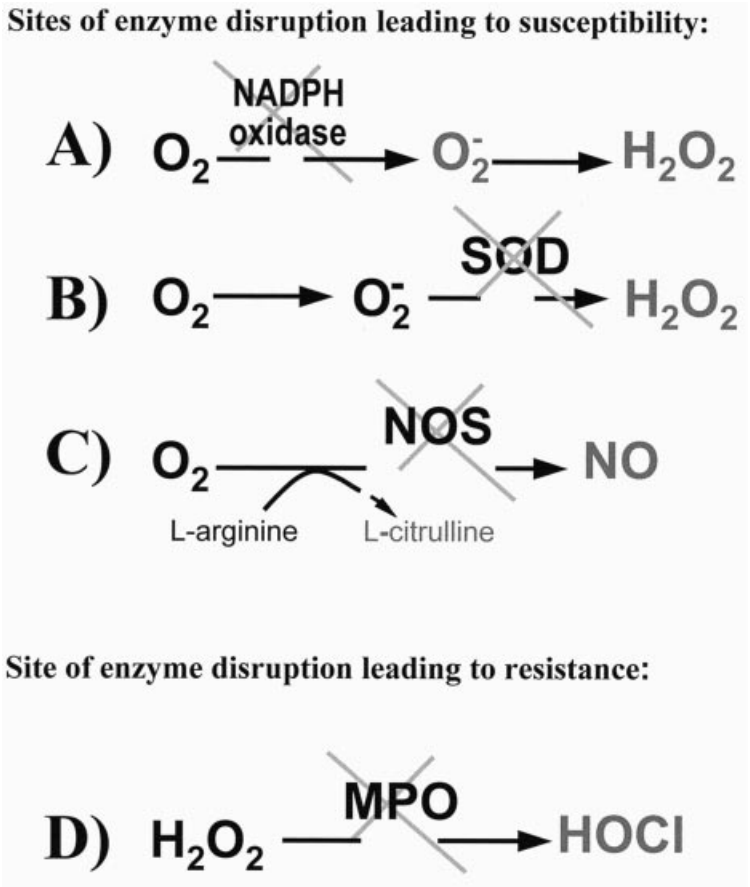

Fig. 3. Hypothetical alterations in biochemical pathways known to be involved in *Schistosoma mansoni* killing by *Biomphalaria glabrata* haemocytes. Defects that would promote susceptibility to *S. mansoni* include decreased expression or reduced enzyme activity of: (A) NADPH oxidase, (B) superoxide dismutase (SOD), or (C) nitric oxide synthase (NOS). (D) Defects in the activity or expression of myeloperoxidase (MPO) would potentially promote resistance to *S. mansoni* by increasing the net production of hydrogen peroxide (H_2O_2).

advantage lies in an increased susceptibility to certain pathogens. If so, then this constitutes a mirror image of the parasite-driven retention of sickle cell genes in human populations that are sympatric with *Plasmodium* spp. In both cases, a mutation that would be detrimental in the absence of a parasite is retained because it provides survival value in parasitized individuals.

WHAT IS THE ROLE OF THE PARASITE IN AVOIDING DESTRUCTION?

First, flukes that parasitize planorbids must be presumed to be insensitive to whatever molecules kill lymnaeid parasites (Sapp & Loker, 2000*a*). However, until such a snail molecule is isolated it will remain unclear what strategy or mechanism is used by the flukes.

A question remains: does a sporocyst play an active role in avoiding destruction by the oxygen-dependent cytotoxic armoury of a snail? Possibilities include strategies that (a) avoid eliciting the respiratory burst of the host's haemocytes, (b) inhibit the host's systems for generating ROS, (c) rapidly scavenge and detoxify products of the respiratory burst, or even (d) drive away the host haemocytes. Any or all of these may be used, and empirical data suggest that each may play a role. For example, relative to (a) avoidance, sporocysts mimic antigens expressed on host cells (Bayne & Stevens, 1983; Yoshino & Bayne, 1983) and acquire host plasma antigens (Bayne, Loker & Yui, 1986). Both of these strategies may reduce the apparent 'foreignness' of the parasite's surface (Damian, 1987), making it more difficult for the host to recognize the parasite. Relative to (b) inhibition, products secreted by schistosomes as they transform from miracidia into sporocysts (their excretory-secretory products; Lodes, Connors & Yoshino, 1991) alter the metabolism of host haemocytes (Loker, Bayne & Yui, 1986; Loker, Cimino & Hertel, 1992; Coustau & Yoshino, 1994), reducing both motility (Lodes & Yoshino, 1990) and phagocytosis (Connors & Yoshino, 1990), altering the profile of secreted molecules (Yoshino & Lodes, 1988) and modulating their production of ROS (Connors, Lodes & Yoshino, 1991). Relative to (c) scavenging and detoxifying, schistosomes express two enzymes capable of scavenging ROS – superoxide dismutase

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and glutathione peroxidase (Mei & LoVerde, 1997), and release proteases that degrade snail proteins (Yoshino *et al*. 1993). Finally, relative to (d) haemocyte repulsion, haemocytes that are attached and spread out on glass will round up if an echinostome sporocyst comes to reside nearby (Adema *et al*. 1994*a*). This ability appears non-functional for *S. mansoni* in *B. glabrata*, however, since no such effect on haemocytes was evident when these were evaluated *in vitro* (Sapp & Loker, 2000*b*). Furthermore, when sporocysts are brought into close contact with susceptible strain haemocytes *in vitro*, the parasite can become enveloped in a mass of haemocytes yet suffers no harm (Boehmler, Fryer & Bayne, 1996; Hahn *et al*. 2001*a*). It is clear that sporocysts smothered in haemocytes will survive well so long as the haemocytes come from susceptible snails. One is forced, then, to consider survival strategies other than repulsion of haemocytes.

One further strategy that parasites could use to protect themselves from host aggression exploits putative inhibitory receptors on host leukocytes. The notion draws on recent evidence that the failure of certain cytotoxic cells to respond aggressively to host (self) cells is due the expression, on the host cells, of ligands for receptors that switch off the aggressive machinery of the killer cell (Ljunggren & Karre, 1990). If such mechanisms exist at this level of phylogeny (as they surely must), then perhaps host antigens mimicked by a parasite may include such ligands. This idea has been examined in the *S. mansoni* – *B. glabrata* parasitism (Loker & Bayne, 2001), but no supporting evidence was found, perhaps because of reliance on reagents (antibodies) developed for use with mammalian systems.

CONCLUDING COMMENTS

Practically a decade into the genomic era, concrete hypotheses are emerging to account for the outcome when a fluke larva enters the haemocoel of a snail. Compared to 1971 when Wright published *Flukes and Snails*, knowledge of the mechanisms used by snails to attack parasites has expanded particularly in the area of oxygen-dependent mechanisms. Furthermore, knowledge of mechanisms used by parasites to survive in immunologically hostile body compartments has been extended to include enzymes that scavenge reactive oxygen species, and the extent of the cross-talk between the two species is now more fully appreciated. The road to understanding continues to reward the persistent investigator. In view of the extent of human suffering that results from flukes and their kin, it can be confidently predicted that progress in this area will continue. Perhaps the understanding will be complete before yet another generation of parasitologists complete their research careers focused on flukes and snails.

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