Chapter 7
Immunobiology of *Biomphalaria*–Trematode Interactions

Timothy P. Yoshino and Christine Coustau

Abstract As the exclusive intermediate host of the human blood fluke *Schistosoma mansoni*, species of the snail *Biomphalaria*, especially *Biomphalaria glabrata*, have been the subjects of numerous studies focused on the immunobiology of parasite–host interactions. With the recent applications of molecular, genomic, proteomic and glycomic approaches to the study of *Biomphalaria*'s immune response to schistosomes and other trematode species, there is now accumulating a wealth of information that is beginning to address the mechanisms underlying these complex parasite–host associations. In this chapter, we attempt to broadly review our past understanding of *Biomphalaria* immunity and integrate recent information on the cellular and molecular events surrounding initial immune responses to larval trematode infections, the consequences of immune interactions and counter-immune strategies used by these parasites.

7.1 Introduction

7.1.1 Why Study Biomphalaria?

Freshwater snails of the genus *Biomphalaria* would be considered just another "pond snail" were it not for the fact that species of this group represent the obligate intermediate host for the human blood fluke *Schistosoma mansoni*, a major causative agent of hepatosplenic schistosomiasis affecting an estimated 83 million people in 54 countries worldwide (Crompton 1999). A cursory PubMed search (http://www.ncbi.nlm.nih.gov/) of the terms "*Biomphalaria*" or "*Australorbis*" (its former taxonomic name) revealed 2,374 and 2,522 published works, respectively, dating back to the late 1940s. The first listed publication for *Biomphalaria* entitled "Susceptibility

T.P. Yoshino (✉)
Department of Pathobiological Sciences, University of Wisconsin,
Madison, WI 53706, USA
e-mail: yoshinot@svm.vetmed.wisc.edu

R. Toledo and B. Fried (eds.), Biomphalaria *Snails and Larval Trematodes*,
DOI 10.1007/978-1-4419-7028-2_7,

of the snail *Biomphalaria boissyi* to infection with certain strains of *S. mansoni*" was by late Dr. Emile A. Malek, one of the pioneers of modern-day medical malacology (Abdel-Malek 1950). For *Australorbis*, the first listed publication was by von Brand and Files (1947) entitled "Chemical and histological observations of *S. mansoni* infections in *Australorbis glabratus*". Of particular relevance to the present chapter, these earliest research topics addressed the differences among different *Biomphalaria* spp. in their susceptibility to *S. mansoni* infection, and the tissue responses evoked in this snail upon larval infection. After more than 60 years since these investigations were conducted, researchers are still continuing to study the underpinnings of schistosome-snail compatibility including the mechanisms underlying host response to infection. Thus, in large part, these earliest works have played (and continue to play) a significant role in the establishment of contemporary studies of molluscan immunobiology with *Biomphalaria* serving as a model organism.

7.1.2 Biomphalaria/Schistosoma *Compatibility and Immunobiological Studies*

As a direct result of this important connection between *Biomphalaria* and its essential role in human blood fluke transmission, many early studies have also investigated the genetic basis of variation in host–parasite compatibility. They were mostly based on experimental infections of inbred laboratory stocks of *Biomphalaria glabrata* originating from allopatric or sympatric populations, and they demonstrated a substantial polymorphism in compatibility (Richards 1975; Richards and Shade 1987). Genetic studies then demonstrated that both snail susceptibility and schistosome infectivity were heritable and could be selected for in the laboratory (Richards and Shade 1987; Richards et al. 1992; Webster et al. 2004). However, subsequent efforts in elucidating the genetic determinism of resistance revealed a variety of underlying mechanisms ranging from a single major locus to a potentially high number of loci (Richards 1975; Richards et al. 1992; Webster and Davies 2001). In addition, snail strains that were naturally incompatible or selected for high incompatibility with one particular schistosome strain were often highly compatible with other schistosome strains (Richards and Shade 1987; Webster and Woolhouse 1998; Webster et al. 2004). These complex results support the idea that *B. glabrata*/*S. mansoni* compatibility relies on complex genotype-by-genotype interactions (Basch 1975; Theron et al. 2008), where the success or failure of infection of an individual snail by an individual parasite larvae would depend on the concordance or discordance of potentially numerous host and parasite genes (and their products). The random loss or fixation of alleles at loci that participate in this genotype-by-genotype interaction may largely explain the diversity in compatibility status or compatibility determinism observed in the laboratory (Theron et al. 2008).

Although compatibility in the *B. glabrata*/*S. mansoni* system appears to be a joint trait of the host and parasite, the terms "susceptibility/resistance" can be used

when focusing on variation in compatibility among different hosts toward the same strain of parasite. Similarly, the term "infectivity" refers to parasite phenotypes measured against the same strain of host. Host and parasite laboratory strains differing in their resistance/susceptibility or infectivity status, respectively, have been particularly useful for functional approaches investigating snail immunity and trematode immune evasion processes. A vast body of literature has accumulated on the fundamental mechanisms of the immune defense system in this snail that represents an important comparative model for other lophotrochozoan invertebrates (Loker et al. 2004; Raghavan and Knight 2006). In the following review, we attempt to summarize broadly our current knowledge of the immune defense system of *Biomphalaria* spp., specifically its role in regulating interactions with larval trematodes. Although overlap with previous reviews of this topic (Loker and Bayne 1986; Bayne and Yoshino 1989; Yoshino and Vasta 1996; Adema and Loker 1997; Bayne 2009; Coustau et al. 2009) is unavoidable, we attempt to integrate recent information on the molecular events surrounding initial establishment of snail–trematode relationships and immune–counterimmune interactions involved in continued maintenance of larval infections.

7.2 Back to Basics: Components of *Biomphalaria* Innate Immunity

Like other gastropod molluscs, the internal defense or immune system of *Biomphalaria* is composed of both cellular and humoral components, which may act either alone or in concert with each other to recognize and respond to invading microbes or parasites (Yoshino et al. 2001; Lockyer et al. 2004a; Loker et al. 2004; Bayne 2009). Circulating phagocytic cells, termed hemocytes or amebocytes, are found in the hemolymph of the snail and represent the primary effector cells involved in host reactions to invading pathogens, including larval trematodes. The soluble portion of hemolymph, termed plasma (Bayne et al. 1980a), serves as the hemocyte "transportation" system and contains humoral immune factors functioning to facilitate/enhance cell-mediated responses or alone, as immune effectors directly interacting with and eliminating pathogens.

In the last decade, considerable efforts have been made to start characterizing genes or gene products involved in *B. glabrata* immune responses, thanks to the development of methodologies allowing identification and/or comparisons of expressed genes in nonmodel organisms. Gene discovery studies have incorporated various technical approaches including differential display reverse transcriptase, suppression subtractive hybridization, random sequencing of ESTs or ORESTES, and gene expression profiling through microarray studies. Regardless of the methodology used, theses studies have focused on identifying transcripts from circulating hemocytes (Mitta et al. 2005), transcripts differentially expressed before and after an immune challenge (Miller et al. 2001; Raghavan et al. 2003; Lockyer et al. 2000, 2004b, 2007; Nowak et al. 2004; Guillou et al. 2007a; Hanelt et al. 2008),

or differentially expressed transcripts in snails differing in their trematode susceptibilities (Schneider and Zelck 2001; Bouchut et al. 2006a, 2007; Lockyer et al. 2008; Adema et al. 2010; Ittiprasert et al. 2010). Proteomic studies also have been conducted with the aim of characterizing *B. glabrata* proteins that were differentially represented in the plasma or hemocytes from parasite-susceptible and -resistant *B. glabrata* (Vergote et al. 2005; Bouchut et al. 2006b). Taken together, these studies have yielded a multitude of immune-relevant candidates belonging to several major functional categories (see Bayne 2009 ; Coustau et al. 2009 for review) such as genes involved in nonself recognition, cell–cell or cell–matrix adhesion, proteases and protease inhibitors, antimicrobial proteins, immune regulators, antioxidants, as well as components of the three major signaling pathways involved in immune responses (Toll, Imd, MAPK). Thus, although it is clear that the genetic machinery exists in *Biomphalaria* for "building" an effective system of internal defense, the challenge now is to incorporate this vast genetic information into a unified picture of the mechanisms underlying cellular immune recognition and reactivity to invading trematode larvae and other pathogens.

7.2.1 Cellular Components

Hemocytes represent the primary effector cells comprising the snail's internal defense system (Fig. 7.1). Based on the morphology of adherent hemocytes under in vitro conditions, *B. glabrata* hemocytes were initially reported as having two

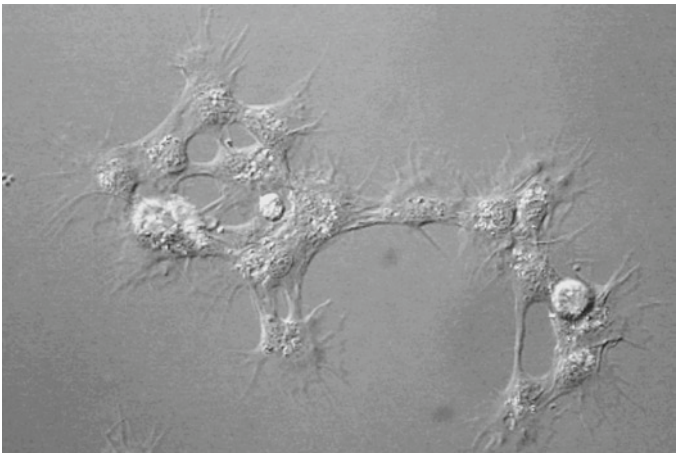

Fig. 7.1 Photomicrograph of live hemocytes of *Biomphalaria glabrata* adhering to and spreading over a glass coverslip surface. Circulating granular hemocytes (granulocytes) represent the primary effector cells involved in the snail's internal defense including parasitic encapsulation reactions. (Nomarski DIC)

distinctive cell types; granulocytes and nongranular hyalinocytes (Cheng 1975; Cheng and Auld 1977), although more recently, variations in the degree of granularity (Martins-Souza et al. 2009), cell size (Matricon-Gondran and Letocart 1999; Martins-Souza et al. 2009), ultrastructural features (Jeong et al. 1983; Matricon-Gondran and Letocart 1999), and cell surface/biochemical markers (Granath and Yoshino 1983; Yoshino and Granath 1985; Martins-Souza et al. 2006) have prompted modifications of the snail's cell classification scheme. Regardless, it is clear that *Biomphalaria* hemocytes represent a morphologically and functionally heterogeneous population(s) whose cellular composition can change with snail age, external factors (e.g., infection), and assay methodology.

Production of hemocytes in *B. glabrata*, and presumably other *Biomphalaria* spp., appears to take place primarily in an "amebocyte-producing organ" (APO; Lie et al. 1975; Jeong et al. 1983) located in the anterior pericardial wall separating the pericardial sac from the mantle cavity, although other sites of blood cell formation also have been proposed (Souza and Andrade 2006). The APO is responsible for maintaining steady-state populations of circulating cells and, importantly, is responsive to introduction of foreign invaders or substances. For example, hematopoeitic activity has been shown to be specifically stimulated by infection with selected trematode species/strains (Lie et al. 1976) or by injection of various foreign materials including schistosome larval extracts (Sullivan et al. 2004; Sullivan 2007) or cell signal-activating agents (Salamat and Sullivan 2009) (Fig. 7.2).

7.2.2 Immune Recognition

Discrimination of "self" from "nonself" by circulating hemocytes of *Biomphalaria* is believed to be accomplished through pattern recognition receptors (PRRs; Janeway and Medzhitov 2002), in which "invariant" cell-associated or soluble receptors bind to fixed foreign chemical structures, referred to as pathogen-associated molecular patterns or PAMPs (Janeway 1989). However, recent discovery of a family of highly diversified pathogen-binding proteins, the fibrinogen-related proteins or Freps (Adema et al. 1997; Zhang et al. 2004), now provides new evidence that *Biomphalaria,* and other snail species (Zhang et al. 2009), possess a structurally and functionally related group of divergent molecules with potential wide-spread pathogen-binding activities (Zhang et al. 2008). Together, with similar findings in arthropod and echinoderm species (e.g., Brites et al. 2008; Buckley et al. 2008; Dong and Dimopoulos 2009; Schmucker and Chen 2009), the notion of invertebrate PRRs as an "invariant" system of immunorecognition is certainly being challenged (see Sect. 7.2.3). Whether "invariant" or "highly diversified", PRR binding to their corresponding PAMPs at the hemocyte surface is presumed to generate intracellular molecular signals that result in a variety of hemocyte responses. Chief among these responses are (a) phagocytosis – internalization and destruction of small particles (e.g., microbes, protozoan parasites), (b) encapsulation – multicellular

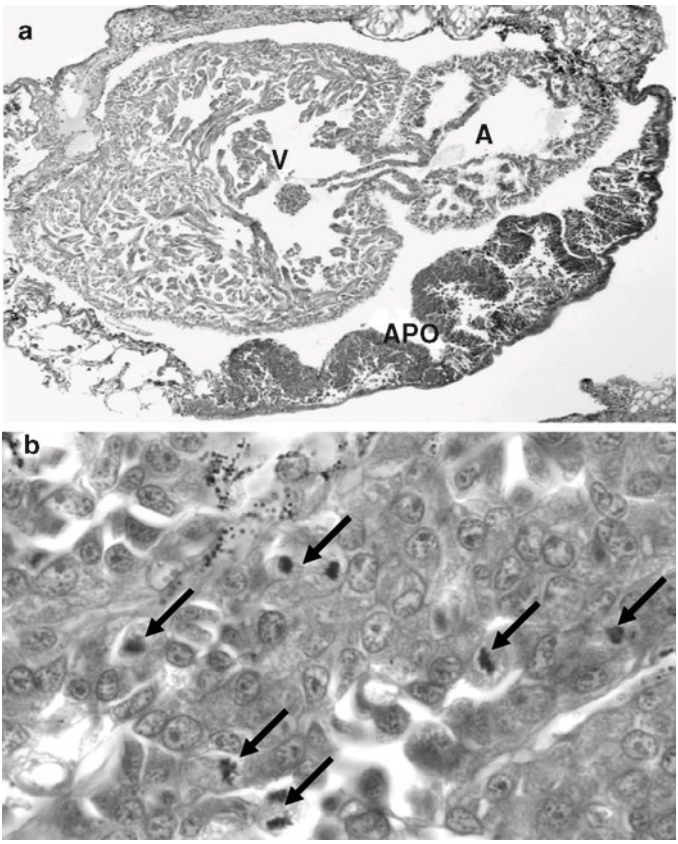

Fig. 7.2 Histological sections of the amebocyte-producing organ (APO), a major source of circulating hemocytes in *Biomphalaria glabrata*. This organ is located in the anterior pericardial wall that forms part of the pericardial sac surrounding the heart (*V* ventricle; *A* atrium) (**a**). Stimulation by specific larval trematode infection or injection with the parasite extracts may result in hyperplastic expansion of this tissue due to increased mitotic activity of hemocyte progenitor cells (**b**, *arrows*). Photomicrographs kindly provided by Dr. John T. Sullivan (Univ. of San Francisco)

reactions in response to foreign bodies or organisms too large to be phagocytosed, and (c) cytotoxicity – extracellular killing of foreign cells or tissue grafts by contact cytolysis. Although phagocytosis represents an extremely important hemocyte function for eliminating microbial infections in *Biomphalaria* and other gastropods (Michelson 1975; Bayne and Fryer 1994; Yoshino and Granath 1985; Martins-Souza et al. 2009), emphasis in this chapter will be given to the encapsulation/cytotoxic responses of hemocytes due to their role in immune defense against larval trematode infections. The role of hemocytes in immune encapsulation of trematodes will be covered in detail later in this chapter.

7.2.3 Cell-Associated and Plasma-Associated Pattern Recognition Receptors

What do we currently know about pattern recognition receptors (PRRs) in *Biomphalaria*? In the context of this chapter, the designation "PRR" is functionally defined as a hemocyte molecule (usually a protein) that binds to constitutively expressed (fixed) structures displayed by the parasite, and elicits in the cell either directly (through a membrane-associated PRR) or indirectly (through soluble PRRs) immune-related responses. In *B. glabrata*, hemocyte-associated adhesion-type molecules, including α and ß integrin-like proteins, cadherins, dermatopontins, and matrilin-like proteins (Davids and Yoshino 1998, 1999; Mitta et al. 2005; Bouchut et al. 2006a), may be functioning as "self" recognition receptors promoting cellular interactions with host extracellular matrix, cytoskeletal or snail plasma proteins, and thereby influencing hemocyte behavior such as cell migration, chemotactic targeting, or cell–cell adhesion during encapsulation or wound healing responses. Other *Biomphalaria* PRRs with known or presumed microbial- or larval trematode-binding capabilities include short- and long-form peptidoglycan recognition proteins (PGRPs), a gram-negative bacterial binding protein (GNBP) (Zhang et al. 2007), LPS-binding proteins/bactericidal permeability increasing proteins (LBP/BPIs) (Mitta et al. 2005; Guillou et al. 2007a), and the large family of fibrinogen-related proteins or Freps (Adema et al. 1997; Zhang et al. 2004, 2008). Unfortunately, except in the cases of ß integrins and selected Frep members (Stout et al. 2009), these adhesion molecules in *Biomphalaria* were identified solely via transcriptomic studies, and therefore little functional data are available for these proteins in this snail, including their role(s) in mediating pathogen recognition. Structurally, however, Zhang et al. (2007) showed that while the GNBP gene appeared to encode a secreted molecule, the three long-form PGRPs lack a signal peptide and thus are presumed to be intracellular in their action. These results are consistent with *Drosophila melanogaster* PGRPs in which the *Dm*PGRP-LE gene encodes three isoforms including two truncated forms, none of which have a signal peptide. Interestingly, these *Dm*PGRP-LE can function by two distinct mechanisms, either as extracellular or intracellular receptors (Kaneko et al. 2006). Based on this information, it seems likely that *Biomphalaria* PGRPs also differ in their location and function (Zhang et al 2007), and future studies are clearly needed to determine whether these PGRPs can function as extracellular receptors.

One class of recognition receptors believed to be of particular importance in initial hemocyte interactions with larval trematodes are the nonenzymatic carbohydrate-binding proteins known as lectins (Endo et al. 2006; Vasta et al. 2007). Given that complex carbohydrates (CHOs) represent major components of the larval tegument (Lehr et al. 2007, 2008; Peterson et al. 2009) and glycoproteins released during miracidial transformation (Wu et al. 2009), it has been hypothesized that a CHO-based recognition system may be pivotal in determining hemocyte responsiveness and ultimately the compatibility phenotype in *B. glabrata*/*S. mansoni* systems (Yoshino and Vasta 1996; Adema and Loker 1997; Loker et al. 2004; Bayne 2009).

This notion is supported by functional data demonstrating involvement of larval CHO in hemocyte-mediated cytotoxicity and phagocytosis (Boswell and Bayne 1985; Fryer et al. 1989), induction of reactive oxygen species (ROS) production in hemocytes (Hahn et al. 2000; Lacchini et al. 2006; Zelck et al. 2007; Humphries and Yoshino 2008), and in vitro adherence of host plasma or hemocyte proteins to the *S. mansoni* sporocyst tegument or released proteins (Johnston and Yoshino 1996, 2001; Castillo and Yoshino 2002; Castillo et al. 2007).

Prominent among lectin-based PRR proteins in *Biomphalaria* are the previously mentioned Freps. This is a highly diversified molecular family comprised of 314 unique gene sequences encoding >200 different proteins (Stout et al. 2009). The rapid and selective upregulation of specific Frep transcripts in response to parasite challenge in resistant snails and the ability of Freps to precipitate larval secretory proteins may be functionally tied to the snail's antiparasite response. This response appears to be quite selective, as only certain Frep members are stimulated/upregulated in reaction to a given trigger (e.g., differing responses to schistosome vs. echinostome infections; Zhang et al. 2008). However, the precise mechanisms of interaction between Freps, hemocytes, and parasite larvae, and how such interactions are related to susceptibility status of a given snail–trematode association still remain to be answered in this system (Bayne 2009).

In addition to Freps, other lectins have been identified in *B. glabrata* including selectin-like proteins (Duclermortier et al. 1999; Guillou et al. 2004), a C-type lectin related to mannose-binding protein (EST only; Bouchut et al. 2007), and a tandem-repeat galectin (Yoshino et al. 2008). The selectins, galectin, and Freps appear to be produced in hemocytes, but only the galectin has been shown to be associated with the hemocyte surface membrane. In functional studies, both recombinant galectin and certain native Freps bind to trematode antigens (CHOs), but differ in their primary sugar-binding specificities (gal/lac/galNAc for galectin vs. fuc for Freps) and divalent cation-dependencies (Adema et al. 1997; Yoshino et al. 2008). In summary, although the in vivo functions of these hemocyte-associated lectins are still a matter of conjecture, current evidence supports a central role of lectin-like PRRs in parasite recognition and initiation of anti-parasite responses: (1) they are synthesized in, and for some, expressed on the surface of hemocytes, (2) they are able to bind/recognize larval CHOs, (3) expression of some are selectively induced by pathogen-challenge, and (4) they possess significant gene homology with vertebrate lectins with known immune function.

7.2.4 Other Immune-Related Plasma Components

In addition to soluble PRRs, *Biomphalaria* plasma contains other proteins with potential immune activity. For example, hydrolytic/cytolytic enzymes such as lysozyme, acid phosphatases, or proteinases (Cheng et al. 1978; McKerrow and Doenhoff 1988; Cheng and Dougherty 1989; Bouchut et al. 2007) or putative antimicrobial proteins (Mitta et al. 2005; Guillou et al. 2007a; Hanelt et al. 2008)

also may be responsible for pathogen killing. Moreover, glycanases such as identified endo-ß mannanases 1 and 2 in *B. glabrata* plasma (Vergote et al. 2005) could function to modify CHO structures at the larval surface rendering the parasite recognizable by lectin PRRs.

7.3 Immune Interactions Between Trematodes and *Biomphalaria*

7.3.1 Encapsulation and Larval Killing In Vivo and In Vitro

In snails exhibiting a resistance phenotype hemocytic encapsulation represents their primary response to invasion by parasitic helminths (Bayne et al. 2001; Bayne 2009; Carton et al. 2005). Typically this involves circulating hemocytes being attracted to and infiltrating the area surrounding the parasite, followed by the formation of a multilayered cellular capsule leading eventually to larval death (Sullivan and Richards 1981; Loker et al. 1982). As mentioned earlier, the capacity of *Biomphalaria* hemocytes to recognize and encapsulate various trematode species, most notably *S. mansoni* and several species of echinostomes, is genetically determined, which has led to the isolation and cultivation of a number of genetically inbred *B. glabrata* strains selected for their susceptibilities to a given isolate of *S. mansoni* or *Echinostoma* spp. (Langand and Morand 1998; Ataev and Coustau 1999; Lewis et al. 2001). Although such inbreeding greatly reduces the genetic variability seen in natural populations (Lockyer et al. 2004a; Theron et al. 2008), these inbred snail lines continue to serve as valuable research resources for investigating host–parasite immune mechanisms under controlled laboratory conditions.

7.3.1.1 Methods of Investigating Immune Interactions

In addition to the establishment of inbred *B. glabrata* strains differing in parasite compatibilities, several other important advancements in the study of hemocyte-mediated encapsulation responses have been made. Of particular significance is the development of in vitro methods for manipulating both the relevant early larval stages of trematode development and elements of the host's immune system. These included the axenic isolation of free-living miracidia of *S. mansoni* and their induced transformation to the first intramolluscan developmental stage, the primary or mother sporocyst (Voge and Seidel 1972), and, based on the manipulation of sporocysts in culture, the design and development of an in vitro assay for evaluating snail hemocyte–sporocyst interactions at the cellular and molecular levels (cell-mediated cytotoxicity or CMC assay; Bayne et al. 1980a, b) (Fig. 7.3). Since these initial reports, there have been many refinements in the cultivation methods and media for maintaining both trematode larvae and snail cells (Ivanchenko et al. 1999;

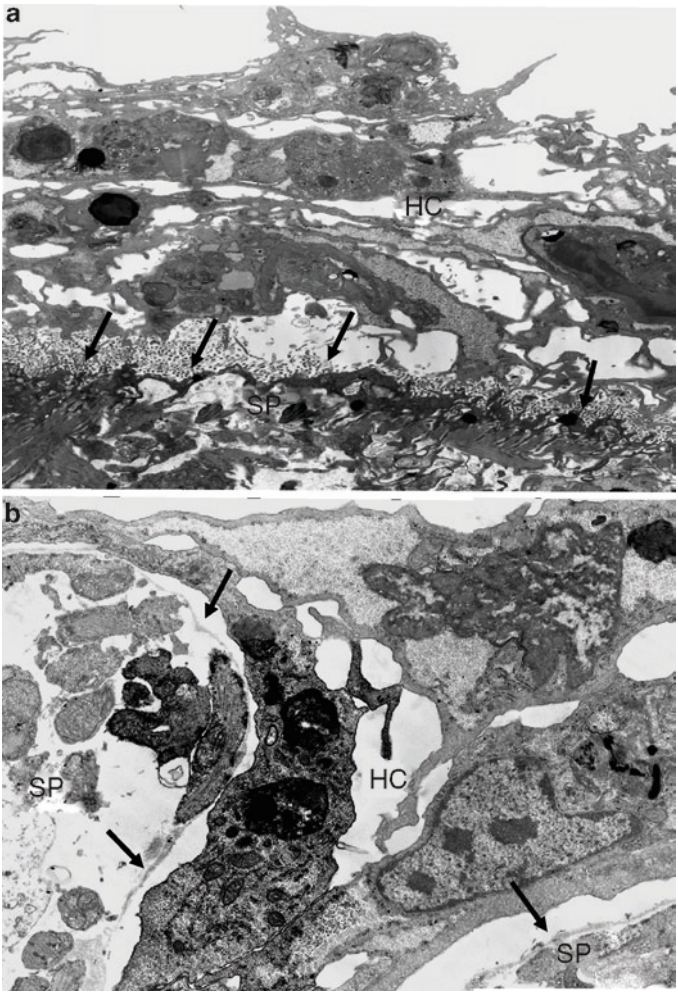

Fig. 7.3 Electron photomicrographs of hemocytic encapsulation of *Schistosoma mansoni* primary sporocysts (SP) by circulating hemocytes (HC) from a schistosome-susceptible (**a**) and -resistant (**b**) strain of *Biomphalaria glabrata*. After 24 h of in vitro hemocyte-sporocyst cocultivation, note the presence of an intact tegument (*arrows*) at the interface with susceptible snail hemocytes (**a**), compared to complete destruction of the tegument and internal damage associated with sporocysts during resistant snail encapsulation reactions (**b**). *Arrows* in panel (**b**) point to the basal lamina, normally located just proximal to the larval tegument, which is missing

Bixler et al. 2001), as well as adaptations of the CMC assay (Bayne 2009) to explore the basic mechanisms underlying *Biomphalaria* susceptibility and resistance to trematode infections.

Another important discovery significantly contributing to the schistosome-snail research field was isolation of the first, and presently only, molluscan cell line from 5-day old embryos of *B. glabrata* (Hansen 1976). The Bge (*B. glabrata* embryonic)

cell line has been an invaluable tool in investigations of snail–trematode interactions by significantly promoting in vitro *S. mansoni* larval development from the miracidium through multiple daughter sporocyst (Yoshino and Laursen 1995) and cercarial (Ivanchenko et al. 1999) stages in coculture experiments. Due to its unique capacity to "nonlethally" encapsulate *S. mansoni* sporocysts Bge cells have been used to investigate adhesion receptors (Duclermortier et al. 1999; Castillo and Yoshino 2002; Castillo et al. 2007), cell signaling pathways (Humphries et al. 2001; Humphries and Yoshino 2006), influence of parasite ESP on snail cell gene expression (Coustau et al. 2003) and snail cell influences on larval *S. mansoni* gene expression (Coppin et al. 2003; Vermeire et al. 2004). Although not ontologically related to hemocytes, the behavioral and molecular attributes shared between these cell types have provided important insights into potential mechanisms involved in larval immune responses (Yoshino et al. 1999; Lockyer et al. 2004a).

7.3.2 Recognition and Initial Triggering of Encapsulation Responses

As alluded to earlier, miracidial penetration of the host snail is accompanied by protein secretion from the lateral and apical penetration glands and release of a barrage of larval proteins during the subsequent transformation to the primary sporocyst stage. It is during this time that the chemical signals "announcing" the parasite's presence is conveyed to the host, and to which the host response is initiated. Although previously referred to as excretory-secretory proteins/products (ESP; Lodes and Yoshino 1989; Guillou et al. 2007b), the term larval transformation proteins or LTPs has been suggested to more accurately reflect this complex group of proteins. This was based on recent findings that the majority of identified *S. mansoni* LTPs lacked signal peptides or characteristics of nonclassical secretion, and that a significant source of released proteins appeared to originate from degenerating ciliated epidermal plates (Wu et al. 2009; Peterson et al. 2009). However, regardless of the origins of ESP/LTPs, the fact that many of these proteins, as well as those expressed at the sporocyst tegument, are heavily glycosylated (Nyame et al. 2002; Lehr et al. 2008; Peterson et al. 2009) suggests that terminal CHOs may play a prominent role in determining hemocytic responses to larval infection. Since ESP continues to be used in the literature, for the purpose of this review the terms ESP and LTP are used synonymously.

Recent analyses of specific glycotopes expressed at the tegumental surface of *S. mansoni* sporocysts and in ESP/LTPs indicate an array of fucosylated CHO structures built around the terminal lacdiNAc or LDN [GalNAcß1-4GlcNAcß1] backbone of expressed schistosome oligosaccharides (Peterson et al. 2009). These include F-LDN, LDN-F, F-LDN-F and LDN-DF. What is not known is whether or not these prominent sugar determinants actually serve as ligands for snail hemocyte or plasma PRRs. Using a cell-adhesion assay modified from Bayne et al. (1984), Castillo and Yoshino (2002) found that the binding of Bge cells to *S. mansoni* sporocysts was inhibited by fucoidan, a sulfated poly-fucose, and monoclonal

antibodies to LDN-F, suggesting the involvement of fucosylated sugars in Bge cell–sporocyst binding. Fucoidan also inhibited hemocyte–sporocyst binding interactions (unpublished observation), suggesting a functional association between fucosylated sugar–snail cell-binding interactions. The observation that precipitation reactions involving Frep-*Echinostoma* ESP were inhibited by L-fucose further suggests that Freps may represent candidate PRRs responsible for mediating initial encapsulation reactions involving hemocytes in resistant (R) *B. glabrata* snails, or blocking of hemocyte reactivity in susceptible snails by interaction with selected fucosylated proteins (Adema et al. 1997; see Sect. 7.4.1). Although other candidate molecules as either host PRRs or potential parasite ligands may be functioning in this system, current evidence favors a lectin-CHO-based recognition system operating to regulate parasite–host immune interactions.

7.3.2.1 Immune Signaling Pathways

In order for hemocytes to mount antiparasite responses, receptor–ligand binding must produce an initial stimulus that triggers the transduction of intracellular signals resulting in the eventual activation of target genes and/or proteins responsible for generating hemocytic effector functions. In *Biomphalaria*, as pointed out earlier, CHOs represent an important class of "ligand" molecules with demonstrated capacity to induce a spectrum of cell-mediated reactions including substrate adhesion, phagocytosis, encapsulation, ROS production and the like. However, until only recently have studies been initiated to determine putative signaling pathways linking together receptor–ligand binding and cell effector function. The involvement of Erk-like mitogen-activated protein kinase (Erk-MAPK) and protein kinase C-like (PKC) signaling proteins in regulating in vitro cell adherence and spreading (Humphries et al. 2001) and phagocytosis (Humphries and Yoshino 2003) was first demonstrated in *Biomphalaria* using the Bge cell line as a model system. That hemocytes of *B. glabrata* and a related snail, *Lymnaea stagnalis*, also rely, at least in part, on MAPK/PKC signaling mechanisms during phagocytosis (Humphries and Yoshino 2003; Plows et al. 2005; Zelck et al. 2007) indicates a functional connection between Bge cells and hemocytes regarding the commonality of their signaling pathways. In addition, studies on *Lymnaea* hemocytes have revealed important aspects of intracellular signaling that relate to *Biomphalaria*: (1) signaling pathways (especially PKC/Erk) can be modulated by specific CHOs (Plows et al. 2005; Lacchini et al. 2006) and (2) PKC signaling may represent a primary pathway functionally linking CHO recognition and the generation of hemocyte ROS (Lacchini et al. 2006). Since treatment of *L. stagnalis* hemocytes with schistosome-related CHOs (neoglycoproteins fuc-BSA and gal-BSA) resulted in downregulation of PKC/Erk signaling (Plows et al. 2005), this set the stage for subsequent studies investigating signaling pathways involved in regulating CHO–ROS interactions (Humphries and Yoshino 2008) and the role of larval ESP/LTPs in modulating this system of intracellular signals (see Sect. 7.4.2.4 for further discussion). To date, although still a relatively new area of investigation, evidence favors the involvement of the PKC/MAPK (Humphries and Yoshino 2003, 2006, 2008;

Zelck et al. 2007; Zahoor et al. 2008) and the phosphatidylinositol 3-kinase (PI3-K) (Zelck et al. 2007; Humphries and Yoshino 2008; Salamat and Sullivan 2009) pathways of signal transduction in hemocytes of *B. glabrata*.

Probably due to the fact that little attention has been given to *Biomphalaria* antimicrobial response, information regarding the well-known Toll and Imd immune pathways are scarce. A Genbank database search reveals the existence of a transcript predicted to correspond to NF-kappa B (Genbank accession #FJ711166), a key element in both pathways (Tanji et al. 2007), but to our knowledge this result has not yet been published. Intriguing also is the fact that PGRPs, known to serve as receptor complexes in the IMD pathways (Maillet et al. 2008), have been identified in *B. glabrata* (see Sect. 7.2.3). Although it is likely that Toll- and Imd-related pathways exist in *B. glabrata*, there is no evidence to date suggesting that they may be involved in regulating parasite infection.

7.3.2.2 Consequences of Parasite Recognition: Effector Mechanisms

Depending on the specific intracellular signaling pathway(s) triggered by receptor–ligand binding, hemocytes may respond by either upregulating or downregulating its effector cell activity. As discussed below, "purposeful" downregulating of hemocyte function may represent an important anti-immune mechanism by some trematode species or strains. However, in *Biomphalaria* snails that efficiently fight against a trematode infection, cellular activation usually leads to larval killing. How is this accomplished? Using an in vitro CMC assay, Bayne and colleagues (2001) have demonstrated that ROS, especially H_2O_2, and reactive nitrogen species (RNS) elaborated from hemocytes of *S. mansoni*-resistant (R) strains of *B. glabrata* are responsible for the killing of encapsulated sporocysts. In a series of follow-up studies, summarized in a recent review by Bayne (2009), they have implicated a Cu/Zn-superoxide dismutase (SOD1) as a key enzyme in the oxidative killing activity of R hemocytes based on their experimental findings: (a) SOD1 gene expression and enzyme activity were higher in R hemocytes compared to those of a susceptible (S) *B. glabrata* strain (Goodall et al. 2004), and this correlated directly with greater H_2O_2 productivity in the R strain (Bender et al. 2005); (b) their finding that *B. glabrata* SOD is comprised of three alleles, of which one (SOD1 B allele) was significantly associated with the R snail phenotype (Goodall et al. 2006), and (c) data indicating that SOD1 B allelic expression was greater in R hemocytes and significantly correlated with snail resistance (Bender et al. 2007). Based on their findings, it was suggested that snail differences in SOD1 hemocyte expression is causally linked to the observed susceptibility and resistance in their host–parasite strains system. Clearly the connection between elevated SOD enzyme expression/activity and cytotoxic potential involves the augmentation of H_2O_2 formation from superoxide during the respiratory burst, and eloquently explains the ultimate outcome of R phenotype expression (Bayne 2009).

Whether other effector mechanisms exist in *Biomphalaria* to deal with trematode infections is currently not known. Recent transcriptome analyses of R versus S strain

snails (whole body or hemocytes) reveal a variety of proteases and protease inhibitors that may play a role by directly distrupting tegumental membrane proteins resulting in fatal breaching of the parasite surface, or indirectly by modifying surface proteins or glycoproteins uncovering hidden molecular patterns that are recognized by host hemocytes or plasma PRRs (e.g., Lockyer et al. 2007; Hamlet et al. 2008). Other hydrolytic enzymes are described both within hemocytes and in plasma, but their role in antitrematode responses is unclear (Cheng 1975). Their activity more likely is focused on destruction of microbial invaders that are more sensitive to these lysosomal-type enzymes (Cheng et al. 1978).

7.4 Anti-immune Counter-Measures by Parasites

7.4.1 Mimicry or Immunological Smokescreen

Together with antigenic variation and immune suppression or modulation, molecular mimicry is considered as one of the major process of parasite immune evasion (Damian 1989, 1997). The idea that schistosomes avoid immune recognition partly through molecular mimicry processes originates from early studies showing cross-reactivity between polyclonal antibodies raised against *Biomphalaria* hemolymph and schistosome miracidia and/or sporocysts (Yoshino and Cheng 1978; Yoshino and Bayne 1983). In these studies, both miracidia and in vitro-transformed mother sporocysts were derived in media devoid of snail host material, preventing the possibility that host antigens may have been acquired by the parasite during intra-host development. These results therefore showed that miracidial and sporocysts stages of *S. mansoni* constitutively harbor snail-like antigens (Yoshino and Cheng 1978; Yoshino and Bayne 1983). A reciprocal study confirmed the existence of mimicked antigens, as it showed that antibodies raised against schistosome miracidia cross-reacted with hemocytes or soluble plasma from *B. glabrata* (Bayne and Stephens 1983).

Although the nature of these shared antigens was unknown, previous results pointed out the possible importance of carbohydrates as shared moieties. Investigations using carbohydrate-binding lectins showed that some lectins were binding both parasite larval stages (miracidia and mother sporocysts) and soluble plasma components (Stanislawski et al. 1976; Yoshino et al. 1977). Using a monoclonal antibody to a schistosome oligosaccharide epitope, Dissous et al. (1986) also detected cross-reactivity between glycoproteins on miracidia surface and a 39-kDa glycoprotein from *B. glabrata*. More recently, studies that focused on the structural characterization of *N*-glycans from *S. mansoni* soluble egg antigens confirmed that cross-reacting species represent about 5% of the total glycans (Lehr et al. 2007), further supporting the potential importance of carbohydrate moieties as shared epitopes participating in molecular mimicry in this host–parasite system (Lehr et al. 2008; Peterson et al. 2009). As pointed out by Damian (1989), the concept of molecular mimicry refers both to the fact that parasites or pathogens constitutively express shared moieties with their host (probably through convergent

evolution), and that this molecular sharing benefits the parasite by preventing their recognition as nonself. However, evidence for such a beneficial effect is scarce in the *Biomphalaria-Schistosoma* system, and the question of molecular mimicry as a major schistosome immune evasion process continues to be debated (Yoshino and Boswell 1986; Yoshino and Vasta 1996; Adema and Loker 1997; Bayne 2009).

Another possible mechanism involving glycoproteins participating in schistosome immune protection has been reported recently. As an approach to gain insights into the molecular determinant of *B. glabrata/S. mansoni* compatibility, Roger et al. (2008a) compared the proteomes of two strains of *S. mansoni* that were compatible and incompatible with a particular strain of *B. glabrata.* In their initial investigation, several mucin-like molecules were identified among differentially expressed proteins. Subsequent characterization of this set of proteins revealed a high degree of molecular polymorphism (Roger et al. 2008b) resulting from their being encoded by an extensive multigenic family whose individual members frequently recombine and are transcribed as multiple splice variants (Roger et al. 2008c). Because these polymorphic mucins, designated *Sm*PoMuc, are only expressed in the larval stages that interact with the snail host and are released during miracidial transformation as ESP, it has been suggested that these highly polymorphic and glycosylated proteins potentially may bind snail PRRs, such as snail Freps, thus serving as an immunological smokescreen functioning to overwhelming the snail immune system (Roger et al. 2008b, c). Assuming that CHOs represent the ligands (PAMPs) for lectin-like freps and other lectin PRRs, the diversity of sugar structures associated with larval N- and O-linked glycans (Peterson et al. 2009) provides an even greater repertoire of potential divergent structures for each SmPoMuc. Current efforts to begin identifying the glycan structures associated with larval mucins and other glycoproteins, and their reactive lectin counterparts are now in progress.

7.4.2 Direct Interference with Immune Effector Cell Function

7.4.2.1 Echinostome Versus Schistosome: Mechanisms of Survival

In addition to the possible avoidance of recognition through molecular mimicry or by "overwhelming" the immune system via the release of polymorphic glycoproteins such as the *Sm*PoMuc, larval trematodes also can influence the host cellular response by directly modulating or interfering with hemocyte activity/function. This is most evident in the case of echinostome infections of *B. glabrata* in which it has been shown that hemocytes from infected snails lose adherence and phagocytic capabilities in vitro, as well as their larval encapsulation responsiveness (Loker 1994; Adema and Loker 1997; Humbert and Coustau 2001; Coustau et al. 2009). Early larval elaboration of ESP/LTP appears to be the source of hemocyte-modulating molecules (Loker et al. 1992), providing additional evidence for the importance of these parasite products in the initial establishment echinostome infections. By comparison, *S. mansoni* also appears to modulate hemocyte defense functions,

although *S. mansoni* disruption of *B. glabrata* hemoocyte responses is much less aggressive than interference observed in other trematodes such as echinostomes (Guillou et al. 2007b; Bayne 2009; Coustau et al. 2009).

Past in vitro studies showed that *S. mansoni* sporocysts or their ESP could affect hemocyte motility (Lodes and Yoshino 1990), intracellular protein synthesis (Lodes et al. 1991) and phagocytosis (Fryer and Bayne 1990). At the time of these early studies, the molecular characterization of active ESP factors had not developed beyond determination of the molecular weight of protein fractions, and therefore the identity of putative immunomodulatory factors remained unknown. However, with recent proteomic studies of *S. mansoni* and *E. caproni* ESP/LTP, protein candidates with possible immunomodulating activities are now being identified (Guillou et al. 2007b; Wu et al. 2009). For example, a calreticulin (CRT) has been identified in the ESP proteome of *S. mansoni*. CRTs are well-conserved proteins that control Ca^{2+} homeostasis and are therefore involved in many cellular functions, including the Ca-dependent cellular adhesion and spreading processes. As in a parasitoid wasp, in which CRT has been shown to inhibit host hemocyte spreading, Guillou et al. (2007b) hypothesized that secretion of the *S. mansoni* CRT could modify the extracellular concentration of Ca^{2+} and prevent the hemocyte encapsulation process. In another proteomic study (Wu et al. 2009), several other Ca^{2+}-binding proteins with potential immune modulatory activity were identified. One of particular interest was a calcium-binding dynein light chain sharing significant homology with the fish (*Apinephelus*) and human PIN or protein inhibitor of nitric oxide (NO) synthase, an essential enzyme involved in NO formation and NO-mediated larval killing by *B. glabrata* hemocytes (Hahn et al. 2001b). Other candidate proteins included a phospholipid-binding protein and an annexin, both of which are capable of membrane binding and cell entry, and affecting various cell signaling activities (Wu et al. 2009).

7.4.2.2 Antioxidant Molecules in ESP/LTP Associated with Larval Protection

Parasite survival relies, at least in part, on their ability to detoxify the ROS present within their normal host environment or produced as a consequence of their host's immune response. Trematodes are exposed to ROS throughout their life cycle, including their intramolluscan stages. As mentioned earlier, hydrogen peroxide (H_2O_2) and NO are known to be important in parasite killing (Hahn et al. 2001a, b), and for this reason, a number of complementary studies have investigated the presence or the activity of antioxidant molecules in larval *S. mansoni*. Early investigations have shown that *S. mansoni* ES products/proteins (=LTPs; Wu et al. 2009) released during the miracidium-to-mother sporocyst transformation could inhibit superoxide production in snail hemocytes (Connors and Yoshino 1990). Molecular separation of crude ESP/LTP resulted in the isolation of a 108-kDa polypeptide fraction capable of scavenging both exogenously produced and *B. glabrata* hemocyte-derived superoxide (O_2^-) anions (Connors et al. 1991). In another study, Zelck and von Janowsky (2004) targeted three key antioxidant

enzymes, SOD, glutathione peroxidase (GPx) and glutathione-*S*-transferase (GST), and showed that their transcripts were expressed in the sporocyst stages of *S. mansoni*. Although the potential secretion of these enzymes was not studied, their increase in expression upon in vitro exposure to ROS or to snail hemocytes strongly suggested that they were involved in ROS detoxification (Zelck and von Janowsky 2004). Using a similar approach, Vermeire and Yoshino (2007) targeted three peroxiredoxin genes (Prx1, Prx2, Prx3) that are key enzymes in the glutathione (GSH)-thioredoxin redox pathway (Sayed and Williams 2004). They showed that Prx1 and Prx2 transcripts were upregulated during early *S. mansoni* sporocyst development, and that enzyme proteins were expressed within the apical papillae of miracidia and the sporocyst tegument, as well as in secretions released during in vitro larval transformation. Further evidence that these enzymes may function in the protection of *S. mansoni* sporocysts was provided by the fact that removal of Prx1 and 2 from larval ES products by immunoabsoption significantly reduced its ability to breakdown exogeneous H_2O_2 (Vermeire and Yoshino 2007).

Another series of studies suggesting the importance of antioxidant molecules consisted of proteomic analyses of ESP/LTP released during in vitro transformation of *S. mansoni* miracidia to mother sporocysts (Guillou et al. 2007b; Wu et al. 2009). Guillou and coworkers (2007b) identified three major groups of proteins in the ESP of *S. mansoni* sporocysts: antioxidant enzymes, glycolytic enzymes and calcium-binding proteins. The secreted antioxidant enzymes were Cu/Zn SOD and GST. The Cu–Zn SOD catalyzes the dismutation of superoxide anion (O_2^-) into H_2O_2, and as a result of its peroxidative activity, SOD is able to utilize its own dismutation product, H_2O_2, as a substrate catalyzing further breakdown of H_2O_2 to H_2O and O_2 (Kim and Kang 1997). Thus, it appears that *S. mansoni* Cu/Zn SOD could represent a first line of cellular antioxidant defense through its peroxidative activity, resulting in H_2O_2 inactivation and production of either H_2O/O_2 or •OH (via the Fenton rxn). •OH has been shown to be considerably less toxic than H_2O_2 for sporocysts (Hahn et al. 2001a). A second group of antioxidant enzymes identified in proteomic studies was the GSTs including GST26, GST28 and GSTomega (Guillou et al. 2007b; Wu et al. 2009). GSTs neutralize cytotoxic by-products of lipid peroxidation arising from ROS acting on the cell membrane (Tew and Ronai 1999).

In addition to antioxidant enzymes, *S. mansoni* ESP/LTPs were found to contain a number of glycolytic enzymes, for example, triosephosphate isomerase (TPI), a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and an NAD-dependent malate dehydrogenase, possibly acting as reducing agents. Although the presence of glycolytic enzymes may be surprising in an extracellular environment, these enzymes have been reported in ES products from cercarial or adult schistosomes (Knudsen et al. 2005; Perez-Sanchez et al. 2006). TPI and GAPDH are involved in two successive reactions of the glycolysis involving the reduction of a molecule of NAD^+ to NADH. The NADH generated can be used as a reducing agent to reduce oxidized glutathione (GSSG) accumulating due to the GST activity, thus restoring intracellular pools of reduced glutathione (GSH). These results strongly support the view that these enzymes of glycolysis may be involved in antioxidant stress response by contributing to the synthesis of NADH involved in the glutathione redox cycle

(Kum-Tatt et al. 1975; Guillou et al. 2007b). In addition to the aforementioned antioxidant proteins, Wu et al. (2009) also identified several additional redox/antioxidant molecules in their shotgun proteomics screen including a thioredoxin, H_2O_2-scavenger Prx, and hydroxyacylglutathione hydrolase. This study therefore largely confirmed the existence of the antioxidant proteins reported in previous studies and completed the identification of this parasite's antioxidant arsenal.

7.4.2.3 Role of Endogenous Antioxidant Enzyme Systems Against External Oxidative Stress

Although elaboration of potentially protective antioxidant proteins during the first hours of larval development following snail infection may be achieved by ESP/LTPs release during larval transformation, maintaining an intact and functional system throughout the development within the snail also would be of critical importance. This notion was recently explored in a study by Mourão et al. (2009a) in which RNA interference (RNAi) was used to knockdown transcript and protein expression of several antioxidants in *S. mansoni* sporocysts to determine whether endogenous larval antioxidants functioned to protect parasites from external oxidative stress. Results showed that sporocysts treated with double-stranded (ds)RNA for GST26, GST28, Prx and GPx exhibited higher sensitivity to the toxic effects of sublethal H_2O_2 treatment compared to larvae treated with control green fluorescent protein (GFP) dsRNA. Similarly, antioxidant dsRNA-treated sporocysts were more readily killed by susceptible NMRI hemocytes than GFP dsRNA-treated larvae in in vitro CMC assays, demonstrating for the first time a sporocyst-protective function of endogenous antioxidants against external oxidative stress, including that produced during hemocytic encapsulation reactions.

7.4.2.4 Interference with Signaling Pathways

As discussed previously, larval trematode entry into its snail host may result either in stimulating the immune system (serving as "danger" signals; Bianchi 2007) or suppressing immunity through various hypothetical mechanisms including molecular mimicry (Damian 1989), compatibility polymorphism (Roger et al. 2008c) or by direct modulation of hemocyte reactivity. Although these general hypotheses of parasite–host immune interactions have been recognized, the specific molecular mechanism(s) by which either immune stimulation or suppression occurs is still poorly understood. One hypothesis for parasite-mediated suppression suggests that molecules (mainly glycoproteins) comprising ESP/LTPs released during early intramolluscan development can affect hemocyte function by modulation of cell signaling pathways (Walker 2006). This may be accomplished, in theory, by one of the following two mechanisms: (1) binding to a specific receptor that transmits a negative (downregulating) signal in cells or (2) molecules directly interferring with receptors involved in the cell's signaling network. These notions are based on

earlier findings that ESP from echinostome larvae can directly interfere with *B. glabrata* hemocyte functions including phagocytosis and encapsulation reactions (Loker 1994; Loker et al. 1992; Coustau et al. 2009), and in *S. mansoni*, the inhibition of hemocyte motility and intracellular superoxide production (Lodes and Yoshino 1990; Connors and Yoshino 1990) by in vitro ESP treatment.

More recently, this hypothesis has taken on broader support as a result of experiments designed to assess the effect of ESP/LTPs on various signaling pathway molecules involved in the regulation of ROS and RNS production in hemocytes. For example, Erk and p38 MAPK/PKC-associated H_2O_2 release from hemocytes stimulated by PMA or BSA-gal was significantly reduced in the presence of ESP indicating that parasite products were either nonstimulatory, were preventing peroxide production/release or were metabolizing/scavenging H_2O_2 as it was being released from hemocytes (Humphries and Yoshino 2008). An earlier finding that larval ESP contained the H_2O_2-scavenging proteins Prx 1 and 2 (Vermeire and Yoshino 2007) implies the latter explanation. However, given that secreted schistosome proteins are highly glycosylated (Lodes and Yoshino 1989; Wu et al. 2009), direct binding of CHOs associated with larval ESP to hemocyte receptors also could be transducing a negative or inhibitory signal thus blocking specific biochemical or cellular activities (Walker 2006). Recently, Zahoor et al. (2008) demonstrated that *S. mansoni* ESP and fixed whole sporocysts significantly impaired Erk signaling in susceptible (S) *B. glabrata* hemocytes, but not in those of resistant (R) snails, implying a direct differential effect of ESP/sporocyst binding on the Erk MAPK signaling pathway in cells of R and S strain snails. In a recent follow-up study, production of NO in *B. glabrata* hemocytes was shown to be regulated through the Erk MAPK pathways, and that NO production could be induced in R strain hemocytes, but not those of S snails (Zahoor et al. 2009). Since *S. mansoni* sporocysts have been shown to be sensitive to NO-mediated killing in vitro (Hahn et al. 2001b), the data suggest that component(s) in ESP may be differentially regulating NO-based cytotoxicity through manipulation of the Erk signaling pathway. Current evidence supports the involvement of the PKC-MAPK pathway in signaling hemocyte–larval interactions and its modulation by larval CHOs. However, given the considerable contributions made by investigators to this parasite–host system, a cohesive picture of the linkages between specific CHO recognition by hemocytes, the signals propagated as a consequence of "recognition," and the effector cell response (ROS generation) has yet to be created.

7.5 Conclusions and Future Directions

7.5.1 General Conclusions

Although our current understanding of *Biomphalaria*/*Schistosoma* interactions remains fragmentary, the data accumulated so far provide evidence for highly complex interactions. When considering the extraordinary diversification of both putative

parasite-binding molecules of the host, such as the Freps, and parasite-secreted *Sm*PoMuc proteins, one can only envision the diverse and complex molecular mechanisms underlying the success or failure of an infection. Adding to the complexity of trematode–snail encounters, the importance of posttranslational modifications such as protein glycosylation and phosphorylation, regulatory enzymes such as protease/protease inhibitors and signal transduction networks also must be recognized as essential parts of this host–parasite molecular dialogue. This is particularly true of signaling pathways involved in snail immunity. In contrast to other invertebrates where the importance of the Toll and Imd pathways has clearly been demonstrated, in studies to date on *Biomphalaria,* strongest evidence mainly points to the involvement of the MAPK- and PKC-related pathways in the immune response. Taken together, results of recent studies support the idea that the innate immune system of *Biomphalaria* may differ substantially from what is known in other invertebrate models (e.g., *Drosophila*, *Caenorabditis*, *Manduca*) belonging to the Ecdysozoa phylum. In addition, because most invertebrate immune studies have focused on antimicrobial responses, relatively little is known about antiparasitic responses, especially those underlying regulatory aspects. It is anticipated, however, that continued efforts to elucidate snail–trematode immunobiological interactions will surely provide us with novel and important information on invertebrate immunity and host–pathogen interactions.

7.5.2 Critical Research Areas for Future Progress

With the dawning of the genomic and now postgenomic eras, new technological approaches in molecular biology, cell biology and biochemistry are now providing increased opportunities to address previously intractable questions associated with trematodes and their snail–host interactions. That being the case, however, there are still many methodological approaches and experimental tools that have yet to be developed to ensure continued future progress in this important research field. Here are some examples.

7.5.2.1 Achievements of Genome Sequencing Efforts

The complete *S. mansoni* genome has now been sequenced and partially annotated, and the first comprehensive analysis recently has been published (Berriman et al. 2009). Together with the sequencing of the *Schistosoma japonicum* genome (The Schistosoma japonicum Genome Sequencing and Functional Analysis Consortium et al. 2009), these represent the first completely sequenced genomes from lophotrochozoan species. Although the initial genomic analysis of *S. mansoni* was primarily orientated toward predicting novel drug targets (Berriman et al. 2009), the availability of this comprehensive dataset now paves the way for future postgenomic studies investigating other biological aspects such as host–parasite relationships.

Regarding *Biomphalaria* genome sequencing, efforts were initiated in 2001 by an international consortium (http://biology.unm.edu/biomphalaria-genome/index.html; Raghavan and Knight 2006). With funding support from the National Human Genome Research Institute, a *B. glabrata* (BBO2 Brazilian strain) BAC library was generated (Adema et al. 2006), and the sequencing of the entire *B. glabrata* genome was initiated through a joint UNM-BRI-TIGR effort currently coordinated by the Washington University Genome Sequencing Center (http://genome.wustl.edu/genomes/view/biomphalaria_glabrata/) (Raghavan and Knight 2006).

To our knowledge, genome sequencing at Washington University is still in progress and an anticipated completion date is not known at this time. One of the major difficulties is the large size of *B. glabrata* genome (930 Mbases), and it is anticipated that annotation will require substantial additional time and efforts. However, such data are crucial to future research developments if we are to begin to fully understand the interactions of *Biomphalaria* spp. and *S. mansoni* at the molecular level.

7.5.2.2 Investigate Host–Parasite Interactomes

Because of methodological constraints, investigations have so far focused on *Biomphalaria* immune response or *Schistosoma* immune evasion strategies. One possibly fruitful line of research is now to directly investigate *Biomphalaria* /*Schistosoma* interactomes. Current proteomic facilities are now able to analyze molecular complexes, thereby identifying interactions of specific proteins in complex mixtures (Holzmuller et al. 2008). In addition, genomic, transcriptomic and proteomic data on *Biomphalaria* and *Schistosoma* should soon be exhaustive enough to permit computational searches for predicted protein–protein or protein-CHO interaction networks between host and parasite (Cui et al. 2009) and facilitate interpretation or integration of proteomic results. Such approaches should greatly facilitate the identification of proteins playing a key role in host–parasite molecular dialogue.

7.5.2.3 Development of Tools for Functional Studies

Currently there exists only one molluscan cell line, the Bge cell line from *B. glabrata*. As pointed out earlier, this cell line has been a useful model for studying genes involved in basic cellular mechanisms such as adhesion, CHO-binding interactions, larval encapsulation and the like. Generation of additional snail cell lines, especially those that are ontologically related to hemocytes, would be a valued resource for future studies on snail internal defense mechanisms. In addition, currently there are no cell lines available for any parasitic platyhelminth, including the schistosome species. This lack of a schistosome cell line has greatly hindered development of transgenic/protein expression systems, as well as approaches to address functionality of parasite genes in their interactions with the snail or mammalian host.

Since validation of gene function is a primary aim of molecular/genomic studies, another major drawback for the *Biomphalaria/Schistosoma* research community has long been the unavailability of gene manipulation techniques (Yoshino et al. 2010). The development of RNA interference technology, although promising, still requires further refinement to improve on its sensitivity, knockdown efficacy and reproducibility. RNAi has been successfully used in both *Biomphalaria* (Zhang et al. 2005; Jiang et al 2006) and larval *Schistosoma* (Boyle et al. 2003; Dinguirard and Yoshino 2006; Mourão et al. 2009a). However, as shown in *S. mansoni*, the success of RNAi gene expression knockdown is highly variable and target gene- or DNA sequence-dependent, and accurate interpretation of results requires particular attention to details of experimental design and execution (Mourão et al. 2009b). A significant future achievement would be the ability to create stable transgenic lines of *Biomphalaria* snails carrying specific "knock-in" or "knock-out" gene characteristics. Although the general technology to reach this goal is available, development of the specific methods and approaches applicable to snails are still lacking. Perhaps, incorporating the Bge cell line in the initial development and testing of transgenic approaches (Yoshino et al. 1998) for eventual application to whole animals should be considered.

7.5.2.4 Address the Question of Interaction Polymorphism

One of the major challenges in deciphering the molecular events underlying host–parasite compatibility in natural populations will be to address the question of host and parasite polymorphism. As mentioned in the Introduction section of this chapter, interactions between *Biomphalaria* spp. and *S. mansoni* populations are extremely variable and are likely determined by complex genotype-by-genotype interactions. Future elucidation of molecular interactions underlying compatibility/incompatibility in laboratory strains should, in the future, be validated in natural snail populations exhibiting a diversity of genotypes. Because improvements and refinements in genomic, transcriptomic and proteomic analysis techniques are advancing at an extremely rapid pace, it seems highly probable that, in the near future, host-parasite interactions may be analyzed at the individual level. Previous identification of key protein families using laboratory strains should facilitate targeted investigations exploring their diversity or variability in host and parasite individuals from natural populations.

References

Abdel-Malek E (1950) Susceptibility of the snail *Biomphalaria boissyi* to infection with certain strains of *Schistosoma mansoni*. Am J Trop Med Hyg 30:887–894

Adema CM, Loker ES (1997) Specificity of digenean-mollusc association. In: Fried B, Graczyk TK (eds) Advances in trematode biology. CRC Press, Boca Raton, pp 229–263

Adema CM, Hertel LA, Miller RD, Loker ES (1997) A family of fibrinogen-related proteins that precipitates parasite-derived molecules is produced by an invertebrate after infection. Proc Natl Acad Sci U S A 94:8691–8696

Adema CM, Luo MZ, Hanelt B, Hertel LA, Marshall JJ, Zhang SM, DeJong RJ, Kim HR, Kudrna D, Wing RA, Soderlund C, Knight M, Lewis FA, Caldeira RL, Jannotti-Passos LK, Carvalho Odos S, Loker ES (2006) A bacterial artificial chromosome library for *Biomphalaria glabrata*, intermediate snail host of *Schistosoma mansoni*. Mem Inst Oswaldo Cruz 101 (Suppl 1):167–177

Adema CM, Hanington PC, Lun CM, Rosenberg GH, Aragon AD, Stout BA, Lennard Richard ML, Gross PS, Loker ES (2010) Differential transcriptomic responses of Biomphalaria glabrata (Gastropoda, Mollusca) to bacteria and metazoan parasites, *Schistosoma mansoni* and Echinostoma paraensei (Digenea, Platyhelminthes). Mol Immunol 47:849–860

Ataev GL, Coustau C (1999) Cellular response to *Echinostoma caproni* infection in *Biomphalaria glabrata* strains selected for susceptibility/resistance. Dev Comp Immunol 23:187–198

Basch PF (1975) An interpretation of snail-trematode infection rates: specificity based on concordance of compatible phenotypes. Int J Parasitol 5:449–452

Bayne CJ (2009) Successful parasitism of vector snail *Biomphalaria glabrata* by the human blood fluke trematode *Schistosoma mansoni*: a 2009 assessment. Mol Biochem Parasitol 165:8–18

Bayne CJ, Fryer SE (1994) Phagocytosis and invertebrate opsonins in relation to parasitism. Ann NY Acad Sci 712:162–177

Bayne CJ, Stephens JA (1983) *Schistosoma mansoni* and *Biomphalaria glabrata* share epitopes: antibodies to sporocysts bind host snail hemocytes. J Invertebr Pathol 42:221–223

Bayne CJ, Yoshino TP (1989) Determinants of compatibility in mollusc-trematode parasitism. Am Zool 29:399–407

Bayne CJ, Buckley PM, DeWan PC (1980a) Macrophage-like hemocytes of resistant *Biomphalaria glabrata* are cytotoxic for sporocysts of *Schistosoma mansoni* in vitro. J Parasitol 66:413–419

Bayne CJ, Buckley PM, DeWan PC (1980b) *Schistosoma mansoni*: cytotoxicity of hemocytes from susceptible snail hosts for sporocysts in plasma from resistant *Biomphalaria glabrata*. Exp Parasitol 50:409–416

Bayne CJ, Loker ES, Yui MA, Stephens JA (1984) Immune-recognition of *Schistosoma mansoni* primary sporocysts may require specific receptors on *Biomphalaria glabrata* hemocytes. Parasite Immunol 6:519–528

Bayne CJ, Hahn UK, Bender RC (2001) Mechanisms of molluscan host resistance and of parasite strategies for survival. Parasitology 123:S59–S67

Bender RC, Broderick EJ, Goodall CP, Bayne CJ (2005) Respiratory burst of *Biomphalaria glabrata* hemocytes: *Schistosoma mansoni*-resistant snails produce more extracellular H_2O_2 than susceptible snails. J Parasitol 91:275–279

Bender RC, Goodall CP, Blouin MS, Bayne CJ (2007) Variation in expression of *Biomphalaria glabrata* SOD1: a potential controlling factor in susceptibility/resistance to *Schistosoma mansoni*. Dev Comp Immunol 3:874–878

Berriman M, Haas BJ, LoVerde PT, Wilson RA, Dillon GP, Cerqueira GC, Mashiyama ST, Al-Lazikani B, Andrade LF, Ashton PD, Aslett MA, Bartholomeu DC, Blandin G, Caffrey CR, Coghlan A, Coulson R, Day TA, Delcher A, DeMarco R, Djikeng A, Eyre T, Gamble JA, Ghedin E, Gu Y, Hertz-Fowler C, Hirai H, Hirai Y, Houston R, Ivens A, Johnston DA, Lacerda D, Macedo CD, McVeigh P, Ning Z, Oliveira G, Overington JP, Parkhill J, Pertea M, Pierce RJ, Protasio AV, Quail MA, Rajandream MA, Rogers J, Sajid M, Salzberg SL, Stanke M, Tivey AR, White O, Williams DL, Wortman J, Wu W, Zamanian M, Zerlotini A, Fraser-Liggett CM, Barrell BG, El-Sayed NM (2009) The genome of the blood fluke *Schistosoma mansoni*. Nature 7253:352–358

Bianchi M (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. J Leuk Biol 81:1–5

Bixler LM, Lerner JP, Ivanchenko M, McCormick RS, Barnes DW, Bayne CJ (2001) Axenic culture of *Schistosoma mansoni* sporocysts in low O_2 environments. J Parasitol 87:1167–1168

Boswell CA, Bayne CJ (1985) *Schistosoma mansoni*: lectin-dependent cytotoxicity of hemocytes from susceptible host snails, *Biomphalaria glabrata*. Exp Parasitol 60:133–138

Bouchut A, Roger E, Coustau C, Gourbal B, Mitta G (2006a) Compatibility in the *Biomphalaria glabrata/Echinostoma caproni* model: potential involvement of adhesion genes. Int J Parasitol 36:175–184

Bouchut A, Roger E, Coustau C, Gourbal B, Mitta G (2006b) Compatibility in the *Biomphalaria glabrata/Echinostoma caproni* model: potential involvement of proteins from hemocytes revealed by a proteomic approach. Acta Trop 98:234–246

Bouchut A, Coustau C, Gourbal B, Mitta G (2007) Compatibility in the *Biomphalaria glabrata/ Echinostoma caproni* model: new candidate genes evidenced by a suppressive subtractive hybridization approach. Parasitology 134:575–588

Boyle JP, Wu XJ, Shoemaker CB, Yoshino TP (2003) Using RNA interference to manipulate endogenous gene expression in *Schistosoma mansoni* sporocysts. Mol Biochem Parasitol 128:205–215

Brites D, McTaggart S, Morris K, Anderson J, Thomas K, Colson I, Fabbro T, Little TJ, Ebert D, Du Pasquier L (2008) The Dscam homologue of the crustacean *Daphnia* is diversified by alternative splicing like in insects. Mol Biol Evol 25:1429–1439

Buckley KM, Terwilliger DP, Smith LC (2008) Sequence variations in 185/333 messages from the purple sea urchin suggest posttranslational modifications to increase immune diversity. J Immunol 181:8585–8594

Carton Y, Nappi AJ, Poirie M (2005) Genetics of anti-parasite resistance in invertebrates. Dev Comp Immunol 29:9–32

Castillo MG, Yoshino TP (2002) Carbohydrate inhibition of *Biomphalaria glabrata* embryonic Bge cell adhesion to primary sporocysts of *Schistosoma mansoni*. Parasitology 125:513–525

Castillo MG, Wu XJ, Dinguirard N, Nyame AK, Cummings RD, Yoshino TP (2007) Surface membrane proteins of *Biomphalaria glabrata* embryonic cells bind fucosyl determinants on the tegumental surface of *Schistosoma mansoni* sporocysts. J Parasitol 93:832–840

Cheng TC (1975) Functional morphology and biochemistry of molluscan phagocytes. Ann NY Acad Sci 266:343–379

Cheng TC, Auld KR (1977) Hemocytes of the pulmonate gastropod *Biomphalaria glabrata*. J Invertebr Pathol 30:119–122

Cheng TC, Dougherty WJ (1989) Ultrastructural evidence for the destruction of *Schistosoma mansoni* sporocysts associated with elevated lysosomal enzyme levels in *Biomphalaria glabrata*. J Parasitol 75:928–941

Cheng TC, Guida VG, Gerhart PL (1978) Aminopeptidase and lysozyme activity levels and serum protein concentrations in *Biomphalaria glabrata* Mollusca challenged with bacteria. J Invertebr Pathol 32:297–302

Connors VA, Yoshino TP (1990) In vitro effect of larval *Schistosoma mansoni* excretory-secretory products on phagocytosis-stimulated superoxide production in hemocytes from *Biomphalaria glabrata*. J Parasitol 76:895–902

Connors VA, Lodes MJ, Yoshino TP (1991) Identification of *Schistosoma mansoni* sporocyst excretory-secretory anti-oxidant molecule and its effect on superoxide production by *Biomphalaria glabrata* hemocytes. J Invertebr Pathol 58:387–395

Coppin JF, Lefebvre C, Caby S, Cocquerelle C, Vicogne J, Coustau C, Dissous C (2003) Gene expression changes in *Schistosoma mansoni* sporocysts induced by *Biomphalaria glabrata* embryonic cells. Parasitol Res 89:113–119

Coustau C, Mitta G, Dissous C, Guillou F, Galinier R, Allienne JF, Modat S (2003) *Schistosoma mansoni* and *Echinostoma caproni* excretory-secretory products differentially affect gene expression in *Biomphalaria glabrata* embryonic cells. Parasitology 127:533–542

Coustau C, Gourbal B, Mitta G, Adema C (2009) Echinostomes and snails: exploring complex interactions. In: Fried B, Toledo R (eds) The biology of echinostomes, from the molecule to the community. Springer, New York, pp 35–59

Crompton DWT (1999) How much helminthiasis is there in the world? J Parasitol 85:397–403

Cui T, Zhang L, Wang XZ, He ZG (2009) Uncovering new signaling proteins and potential drug targets through the interactome analysis of *Mycobacterium tuberculosis*. BMC Genomics 10:118

Damian RT (1989) Molecular mimicry: parasite evasion and host defense. Curr Top Microbiol Immunol 145:101–115

Damian R (1997) Parasite immune evasion and exploitation: reflections and projections. Parasitology 115:S169–S175

Davids BJ, Yoshino TP (1998) Integrin-like RGD-dependent binding mechanism involved in the spreading response of circulating molluscan phagocytes. Dev Comp Immunol 22:39–53

Davids BJ, Yoshino TP (1999) Cloning of a ß integrin subunit cDNA from an embryonic cell line derived from the freshwater mollusc, *Biomphalaria glabrata*. Gene 228:213–223

Dinguirard N, Yoshino TP (2006) Potential role of a CD36-like class B scavenger receptor in the binding of modified low-density lipoprotein acLDL to the tegumental surface of *Schistosoma mansoni* sporocysts. Mol Biochem Parasitol 146:219–230

Dissous C, Grzych JM, Capron A (1986) *Schistosoma mansoni* shares a protective oligosaccharide epitope with fresh-water and marine snails. Nature 323:443–445

Dong Y, Dimopoulos G (2009) *Anopheles* fibrinogen-related proteins provide expanded pattern recognition capacity against bacteria and malaria parasites. J Biol Chem 284:9835–9844

Duclermortier P, Lardans V, Serra E, Trottein F, Dissous C (1999) *Biomphalaria glabrata* embryonic cells express a protein with a domain homologous to the lectin domain of mammalian selectins. Parasitol Res 85:481–486

Endo Y, Takahashi M, Fujita T (2006) Lectin complement system and pattern recognition. Immunobiology 211:283–293

Fryer SE, Bayne CJ (1990) *Schistosoma mansoni* modulation of phagocytosis in *Biomphalaria glabrata*. J Parasitol 76:45–52

Fryer SE, Dykes-Hoberg M, Bayne CJ (1989) Changes in plasma opsonization of yeast after isolation of *Biomphalaria glabrata* in small volumes of water. J Invertebr Pathol 54:275–276

Goodall CP, Bender RC, Broderick EJ, Bayne CJ (2004) Constitutive differences in Cu/Zn superoxide dismutase mRNA levels and activity in hemocytes of *Biomphalaria glabrata* (Mollusca) that are either susceptible or resistant to *Schistosoma mansoni* Trematoda. Mol Biochem Parasitol 137:321–328

Goodall CP, Bender RC, Brooks JK, Bayne CJ (2006) *Biomphalaria glabrata* cytosolic copper/zinc superoxide dismutase SOD1 gene: association of SOD1 alleles with resistance/susceptibility to *Schistosoma mansoni*. Mol Biochem Parasitol 147:207–210

Granath WO, Yoshino TP (1983) Characterization of molluscan phagocyte subpopulations based on lysosomal enzyme markers. J Exp Zool 226:205–210

Guillou F, Mitta G, Dissous C, Pierce R, Coustau C (2004) Use of individual polymorphism to validate potential functional markers: case of a candidate lectin BgSel differentially expressed in susceptible and resistant strains of *Biomphalaria glabrata*. Comp Biochem Physiol B 138:175–181

Guillou F, Mitta G, Galinier R, Coustau C (2007a) Identification and expression of transcripts generated during an anti-parasitic response in *Biomphalaria glabrata*. Dev Comp Immunol 31:657–671

Guillou F, Roger E, Mone Y, Rognon A, Grunau C, Theron A, Mitta G, Coustau C, Gourbal BE (2007b) Excretory–secretory proteome of larval *Schistosoma mansoni* and *Echinostoma caproni*, two parasites of *Biomphalaria glabrata*. Mol Biochem Parasitol 155:45–56

Hahn UK, Bender RC, Bayne CJ (2000) Production of reactive oxygen species by hemocytes of *Biomphalaria glabrata*: carbohydrate-specific stimulation. Dev Comp Immunol 24:531–541

Hahn UK, Bender RC, Bayne CJ (2001a) Killing of *Schistosoma mansoni* sporocysts by hemocytes from reisistant *Biomphalaria glabrata*: role of reactive oxygen species. J Parasitol 87:292–299

Hahn UK, Bender RC, Bayne CJ (2001b) Involvement of nitric oxide in killing of *Schistosoma mansoni* sporocysts by hemocytes from resistant *Biomphalaria glabrata*. J Parasitol 87:778–785

Hanelt B, Lun CM, Adema CM (2008) Comparative ORESTES-sampling of transcriptomes of immune-challenged *Biomphalaria glabrata* snails. J Invertebr Pathol 99:192–203

Hansen EL (1976) A cell line from embryos of *Biomphalaria glabrata* Pulmonata: establishment and characteristics. In: Maramorosch K (ed) Invertebrate tissue culture: research applications. Academic, New York, pp 75–99

Holzmuller P, Grebaut P, Brizard JP, Berthier D, Bossard G, Bucheton B, Vezilier F, Chuchana P, Bras-Goncalves R, Lemesre JL, Vincendeau P, Cuny G, Frutos R, Biron DG (2008) "Pathogeno-Proteomics" toward a new approach of host-vector-pathogen interactions. Ann NY Acad Sci 1149:66–70

Humbert E, Coustau C (2001) Refractoriness of host haemocytes to parasite immunosuppressive factors as a putative resistance mechanism in the *Biomphalaria glabrata - Echinostoma caproni* system. Parasitology 122:651–660

Humphries JE, Yoshino TP (2003) Cellular receptors and signal transduction in molluscan hemocytes: connections with the innate immune system of vertebrates. Integr Comp Biol 43:305–312

Humphries JE, Yoshino TP (2006) *Schistosoma mansoni* excretory-secretory products stimulate a p38 signalling pathway in *Biomphalaria glabrata* embryonic cells. Int J Parasitol 36:37–46

Humphries JE, Yoshino TP (2008) Regulation of hydrogen peroxide release in circulating hemocytes of the planorbid snail *Biomphalaria glabrata*. Dev Comp Immunol 32:554–562

Humphries JE, Elizondo L, Yoshino TP (2001) Protein kinase C regulation of cell spreading in the molluscan *Biomphalaria glabrata* embryonic Bg cell line. Biochim Biophys Acta 1540:243–252

Ittiprasert W, Miller A, Myers J, Nene V, El-Sayed NM, Knight M (2010) Identification of immediate response genes dominantly expressed in juvenile resistant and susceptible Biomphalaria glabrata snails upon exposure to *Schistosoma mansoni*. Mol Biochem Parasitol 169:27–39

Ivanchenko MG, Lerner RS, Toumadje B, Allen B, Fisher K, Barnes DW, Bayne CJ (1999) Continuous *in vitro* propagation and differentiation of cultures of the intra-molluscan stages of the human parasite *Schistosoma mansoni*. Proc Natl Acad Sci U S A 96:4965–4970

Janeway CA (1989) Approaching the asymptope? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol 54:1–13

Janeway CA, Medzhitov R (2002) Innate immune recognition. Ann Rev Immunol 20:197–216

Jeong KH, Lie KJ, Heyneman D (1983) The ultrastructure of the amebocyte-producing organ in *Biomphalaria glabrata*. Dev Comp Immunol 7:217–228

Jiang YG, Loker ES, Zhang SM (2006) In vivo and in vitro knockdown of FREP2 gene expression in the snail *Biomphalaria glabrata* using RNA interference. Dev Comp Immunol 30:855–866

Johnston LA, Yoshino TP (1996) Analysis of lectin- and snail plasma-binding glycopeptides associated with the tegumental surface of the primary sporocysts of *Schistosoma mansoni*. Parasitology 112:469–479

Johnston LA, Yoshino TP (2001) Larval *Schistosoma mansoni* excretory-secretory glycoproteins ESPs bind to hemocytes of *Biomphalaria glabrata* (Gastropoda) via surface carbohydrate binding receptors. J Parasitol 87:786–793

Kaneko T, Yano T, Aggarwal K, Lim JH, Ueda K, Oshima Y, Peach C, Erturk-Hasdemir D, Goldman WE, Oh BH, Kurata S, Silverman N (2006) PGRP-LC and PGRP-LE have essential yet distinct functions in the *Drosophila* immune response to monmeric DAP-type peptidoglycan. Nat Immunol 7:715–723

Kim SM, Kang JH (1997) Peroxidative activity of human Cu, Zn-superoxide dismutase. Mol Cell 7:120–124

Knudsen GM, Medzihradszky KF, Lim KC, Hansell E, McKerrow JH (2005) Proteomic analysis of *Schistosoma mansoni* cercarial secretions. Mol Cell Proteomics 4:1862–1875

Kum-Tatt L, Tan IK, Seet AM (1975) A new colorimetric method for the determination of NADH/NADPH dependent glutathione reductase in erythrocytes and in plasma. Clin Chim Acta 58:101–108

Lacchini AH, Davies AJ, Mackintosh D, Walker AJ (2006) ß-1, 3-glucan modulates PKC signalling in Lymnaea stagnalis defence cells: a role for PKC in H2O2 production and downstream ERK activation. J Exp Biol 209:4829–4840

Langand J, Morand S (1998) Heritable non-susceptibility in an allopatric host-parasite system: *Biomphalaria glabrata* (Mollusca)-*Echinostoma caproni* (Platyhelminthes, Digenea). J Parasitol 84:739–742

Lehr T, Geyer H, Maass K, Doenhoff MJ, Geyer R (2007) Structural characterization of N-glycans from the freshwater snail *Biomphalaria glabrata* cross-reacting with *Schistosoma mansoni* glycoconjugates. Glycobiology 17:82–103

Lehr T, Beuerlein K, Doenhoff MJ, Grevelding CG, Geyer R (2008) Localization of carbohydrates common to *Biomphalaria glabrata* as well as to sporocysts and miracidia of *Schistosoma mansoni*. Parasitology 135:931–942

Lewis FA, Patterson CN, Knight M, Richards CS (2001) The relationship between *Schistosoma mansoni* and *Biomphalaria glabrata*: genetic and molecular approaches. Parasitology 123:S169–S179

Lie KJ, Heyneman D, Yau P (1975) The origin of amebocytes in *Biomphalaria glabrata*. J Parasitol 61:574–576

Lie JK, Heyneman D, Jeong KH (1976) Studies on resistance in snails. 4. Induction of ventricular capsules and changes in the amebocyte-producing organ during sensitization of *Biomphalaria glabrata*. J Parasitol 62:286–291

Lockyer AE, Jones CS, Noble LR, Rollinson D (2000) Use of differential display to detect changes in gene expression in the intermediate snail host *Biomphalaria glabrata* upon infection with *Schistosoma mansoni*. Parasitology 120:399–407

Lockyer AE, Jones CS, Noble LR, Rollinson D (2004a) Trematodes and snails: an intimate association. Can J Zool 82:251–269

Lockyer AE, Noble LR, Rollinson D, Jones CS (2004b) *Schistosoma mansoni*: resistant specific infection-induced gene expression in *Biomphalaria glabrata* identified by fluorescent-based differential display. Exp Parasitol 107:97–104

Lockyer AE, Spinks J, Noble LR, Rollinson D, Jones CS (2007) Identification of genes involved in interactions between *Biomphalaria glabrata* and *Schistosoma mansoni* by suppression subtractive hybridization. Mol Biochem Parasitol 151:18–27

Lockyer AE, Spinks J, Kane RA, Hoffmann KF, Fitzpatrick JM, Rollinson D, Noble LR, Jones CS (2008) *Biomphalaria glabrata* transcriptome: cDNA microarray profiling identifies resistant- and susceptible-specific gene expression in haemocytes from snail strains exposed to *Schistosoma mansoni*. BMC Genomics 9:634

Lodes MJ, Yoshino TP (1989) Characterization of excretory-secretory proteins synthesized in vitro by *Schistosoma mansoni* primary sporocysts. J Parasitol 75:853–862

Lodes MJ, Yoshino TP (1990) The effect of schistosome excretory-secretory products on *Biomphalaria glabrata* hemocytes motility. J Invertbr Pathol 56:75–85

Lodes MJ, Connors VA, Yoshino TP (1991) Isolation and functional characterization of snail hemocyte-modulating polypeptide from primary sporocysts of *Schistosoma mansoni*. Mol Biochem Parasitol 49:1–10

Loker ES (1994) On being a parasite in an invertebrate host: a short survival course. J Parasitol 80:728–747

Loker ES, Bayne CJ (1986) Immunity to trematode larvae in the snail *Biomphalaria*. In: Lackie AM (ed) Immune mechanisms in invertebrate vectors. Clarendon, Oxford, pp 199–220

Loker ES, Bayne CJ, Buckley PM, Kruse KT (1982) Ultrastructure of encapsulation of *Schistosoma mansoni* mother sporocysts by hemocytes of juveniles of the 10-R2 strain of *Biomphalaria glabrata*. J Parasitol 68:84–94

Loker ES, Cimino DF, Hertel LA (1992) Excretory-secretory products of *Echinostoma paraensei* sporocysts mediate interference with *Biomphalaria glabrata* hemocyte function. J Parasitol 78:104–115

Loker ES, Adema CM, Zhang SM, Kepler TB (2004) Invertebrate immune systems – not homogeneous, not simple, not well understood. Immunol Rev 198:10–24

Maillet F, Bischoff V, Vignal C, Hoffmann J, Royet J (2008) The *Drosophila* peptidoglycan recognition protein PGRP-LF blocks PGRP-LC and IMD/JNK pathway activation. Cell Host Microbe 15:293–303

Martins-Souza RL, Pereira CA, Martins-Filho OA, Coelho PMZ, Corrêa A Jr, Negrão-Corrêa D (2006) Differential lectin labelling of circulating hemocytes from *Biomphalaria glabrata* and *Biomphalaria tenagophila* resistant or susceptible to *Schistosoma mansoni* infection. Mem Inst Oswaldo Cruz 101:S185–S192

Martins-Souza RL, Pereira CA, Coelho PMZ, Martins-Filho OA, Negrão-Corrêa D (2009) Flow cytometry analysis of the circulating haemocytes from *Biomphalaria glabrata* and *Biomphalaria tenagophila* following *Schistosoma mansoni* infection. Parasitology 136:670–676

Matricon-Gondran M, Letocart M (1999) Internal defenses of the snail *Biomphalaria glabrata.* I. Characterization of hemocytes and fixed phagocytes. J Invertebr Pathol 74:224–234

McKerrow J, Doenhoff MJ (1988) Schistosome proteases. Parasitol Today 4:334–340

Michelson EH (1975) Cellular defense mechanisms and tissue alterations in gastropod molluscs. In: Maramorosch K, Shope RE (eds) Invertebrate immunity. Academic, New York, pp 181–195

Miller AN, Raghavan N, FitzGerald PC, Lewis FA, Knight M (2001) Differential gene expression in haemocytes of the snail *Biomphalaria glabrata*: effects of *Schistosoma mansoni* infection. Int J Parasitol 31:687–696

Mitta G, Galinier R, Tisseyre P, Allienne JF, Girerd-Chambaz Y, Guillou F, Bouchut A, Coustau C (2005) Gene discovery and expression analysis of immune-relevant genes from *Biomphalaria glabrata* hemocytes. Dev Comp Immunol 29:393–407

Mourão MM, Dinguirard N, Franco GR, Yoshino TP (2009a) Role of the endogenous antioxidant system in the protection of *Schistosoma mansoni* primary sporocysts against exogenous oxidative stress. PLoS Negl Trop Dis 3:e550

Mourão MM, Dinguirard N, Franco GR, Yoshino TP (2009b) Phenotypic screen of early-developing larvae of the blood fluke, *Schistosoma mansoni*, using RNA interference. PLoS Negl Trop Dis 38:e502

Nowak TS, Woodards AC, Jung Y, Adema CM, Loker ES (2004) Identification of transcripts generated during the response of resistant *Biomphalaria glabrata* to *Schistosoma mansoni* infection using suppressive subtractive hybridization. J Parasitol 90:1034–1040

Nyame AK, Yoshino TP, Cummings RD (2002) Differential expression of LacdiNAc, fucosylated LacdiNAc, and Lewis x glycan antigens in intramolluscan stages of *Schistosoma mansoni*. J Parasitol 88:890–897

Perez-Sanchez R, Ramajo-Hernandez A, Ramajo-Martin V, Oleaga A (2006) Proteomic analysis of the tegument and excretory–secretory products of adult *Schistosoma bovis* worms. Proteomics 6:S226–S236

Peterson NA, Hokke CH, Deelder AM, Yoshino TP (2009) Glycotope analysis in miracidia and primary sporocysts of *Schistosoma mansoni*: differential expression during the miracidium-to-sporocyst transformation. Int J Parasitol 39:1331–1344

Plows LD, Cook RT, Davies AJ, Walker AJ (2005) Carbohydrates that mimic schistosome surface coat components affect ERK and PKC signalling in *Lymnaea stagnalis* haemocytes. Int J Parasitol 35:293–302

Raghavan N, Knight M (2006) The snail *Biomphalaria glabrata* genome project. Trends Parasitol 22:148–151

Raghavan N, Miller AN, Gardner M, FitzGerald PC, Kerlavage AR, Johnston DA, Lewis FA, Knight M (2003) Comparative gene analysis of *Biomphalaria glabrata* hemocytes pre- and post-exposure to miracidia of *Schistosoma mansoni*. Mol Biochem Parasitol 126:181–191

Richards CS (1975) Genetic factors in susceptibility of *Biomphalaria glabrata* for different strains of *Schistosoma mansoni*. Parasitology 70:231–241

Richards CS, Shade PC (1987) The genetic variation of compatibility in *Biomphalaria glabrata* and *Schistosoma mansoni*. Parasitology 73:1146–1151

Richards CS, Knight M, Lewis FA (1992) Genetics of *Biomphalaria glabrata* an its effect on the outcome of *Schistosoma mansoni* infection. Parasitol Today 8:171–174

Roger E, Gourbal B, Grunau C, Pierce RJ, Galinier R, Mitta G (2008a) Expression analysis of highly polymorphic mucin proteins Sm PoMuc from the parasite *Schistosoma mansoni*. Mol Biochem Parasitol 157:217–227

Roger E, Mitta G, Mone Y, Bouchut A, Rognon A, Grunau C, Boissier J, Theron A, Gourbal BEF (2008b) Molecular determinants of compatibility polymorphism in the *Biomphalaria glabrata/ Schistosoma mansoni* model: new candidates identified by a global comparative proteomics approach. Mol Biochem Parasitol 157:205–216

Roger E, Grunau C, Pierce RJ, Hirai H, Gourbal B, Galinier R, Emans R, Cesari IM, Cosseau C, Mitta G (2008c) Controlled chaos of polymorphic mucins in a metazoan parasite *Schistosoma mansoni* interacting with its intermediate host *Biomphalaria glabrata*. PLoS Negl Trop Dis 211:e330

Salamat Z, Sullivan JT (2009) Involvement of protein kinase C signalling and mitogen-activated protein kinase in the amebocyte-producing organ of *Biomphalaria glabrata* (Mollusca). Dev Comp Immunol 33:725–727

Sayed AA, Williams DL (2004) Biochemical characterization of 2-Cys peroxiredoxins from *Schistosoma mansoni*. J Biol Chem 279:26159–26166

Schmucker D, Chen B (2009) Dscam and DSCAM: complex genes in simple animals, complex animals yet simple genes. Genes Dev 23:147–156

Schneider O, Zelck UE (2001) Differential display analysis of hemocytes from schistosome-resistant and schistosome-susceptible intermediate hosts. Parasitol Res 87:489–491

Souza SS, Andrade ZA (2006) On the origin of the *Biomphalaria glabrata* hemocytes. Mem Inst Oswaldo Cruz 101:S213–S218

Stanislawski E, Renwrantz LR, Becker W (1976) Soluble blood group reactive substances in the hemolymph of *Biomphalaria glabrata* (Mollusca). J Invertebr Pathol 28:301–308

Stout BA, Adema CM, Zhang SM, Loker ES (2009) Biology of FREPs: diversified lectins with fibrinogen-related domains from freshwater snail *Biomphalaria glabrata*. In: Vasta G, Ahmed H (eds) Animal lectins: a functional view. CRC Press Taylor & Francis, Boca Raton, pp 471–491

Sullivan JT (2007) Mitotic responses to injected extracts of larval and adult *Schistosoma mansoni* in *Biomphalaria glabrata*: effects of dose and colchicine treatment. J Parasitol 93:213–215

Sullivan JT, Richards CS (1981) *Schistosoma mansoni*, NIH-SM-PR-2 strain, in susceptible and nonsusceptible stocks of *Biomphalaria glabrata*: comparative histology. J Parasitol 67:702–708

Sullivan JT, Pikios SS, Alonzo AQ (2004) Mitotic responses to extracts of miracidia and cercariae of *Schistosoma mansoni* in the amebocyte-producing organ of the snail intermediate host *Biomphalaria glabrata*. J Parasitol 90:92–96

Tanji T, Hu X, Weber ANR, Ip YT (2007) Toll and IMD pathways synergistically activate an innate immune response in *Drosophila melanogaster*. Mol Cell Biol 27:4578–4588

Tew KD, Ronai Z (1999) GST function in drug and stress response. Drug Resist Updat 2:143–147

The *Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium; Zhou Y, Zheng H, Chen Y, Zhang L, Wang K, Guo J, Huang Z, Zhang B, Huang W, Jin K, Dou T, Hasegawa M, Wang L, Zhang Y, Zhou J, Tao L, Cao Z, Li Y, Vinar T, Brejova B, Brown D, Li M, Miller DJ, Blair D, Zhong Y, Chen Z; Functional genomics analysis: Liu F, Hu W, Wang ZQ, Zhang QH, Song HD, Chen S, Xu X, Xu B, Ju C, Huang Y, Brindley PJ, McManus DP, Feng Z, Han ZG; Sequencing and assembly: Lu G, Ren S, Wang Y, Gu W, Kang H, Chen J, Chen X, Chen S, Wang L, Yan J, Wang B, Li X, Jin L, Wang B, Pu S, Zhang X, Zhang W, Hu Q, Zhu G, Wang J, Yu J, Wang J, Yang H, Ning Z, Berriman M, Wei CL, Ruan Y, Zhao G, Wang S; Paper writing: Liu F, Zhou Y, Wang ZQ, Lu G, Zheng H, Brindley PJ, McManus DP, Blair D, Zhang QH, Zhong Y, Wang S, Han ZG, Chen Z; Project leaders: Wang S, Han ZG, Chen Z (2009) Genome annotation and evolution analysis: The *Schistosoma japonicum* genome reveals features of host-parasite interplay. Nature 460:345–351

Theron A, Coustau C, Rognon A, Gourbière S, Blouin MS (2008) Effects of laboratory culture on compatibility between snails and schistosomes. Parasitology 135:1179–1188

Vasta GR, Ahmed H, Tasumi S, Odom EW, Saito K (2007) Biological roles of lectins in innate immunity: molecular and structural basis for diversity in self/non-self recognition. Adv Exp Med Biol 598:389–406

Vergote D, Bouchut A, Sautiere PE, Roger E, Galinier R, Rognon A, Coustau C, Salzet M, Mitta G (2005) Characterization of proteins differentially present in the plasma of *Biomphalaria glabrata* susceptible and resistant to *Echinostoma caproni*. Int J Parasitol 35:215–224

Vermeire JJ, Yoshino TP (2007) Antioxidant gene expression and function in in vitro-developing *Schistosoma mansoni* mother sporocysts: possible role in self-protection. Parasitology 134:1369–1378

Vermeire JJ, Boyle JP, Yoshino TP (2004) Differential gene expression and the effects of *Biomphalaria glabrata* embryonic Bge cell factors during larval *Schistosoma mansoni* development. Mol Biochem Parasitol 135:153–157

Voge M, Seidel JS (1972) Transformation in vitro of miracidia of *Schistosoma mansoni* and *S. japonicum* into young sporocysts. J Parasitol 58:699–704

Von Brand T, Files VS (1947) Chemical and histological observations on the influence of *Schistosoma mansoni* infection on *Australorbis glabratus*. J Parasitol 33:476–482

Walker AJ (2006) Do trematode parasites disrupt defence-cell signaling in the snail hosts? Trends Parasitol 22:154–159

Webster JP, Davies CM (2001) Coevolution and compatibility in the snail-schistosome system. Parasitology 123:S41–S56

Webster JP, Woolhouse MEJ (1998) Selection and strain specificity of compatibility between snail intermediate hosts and their parasitic schistosomes. Evolution 52:1627–1634

Webster JP, Gower CM, Blair L (2004) Do hosts and parasites coevolve? Empirical support form the *Schistosoma* system. Am Nat 164:S33–S51

Wu XJ, Sabat G, Brown JF, Zhang M, Taft A, Peterson N, Harms A, Yoshino TP (2009) Proteomic analysis of *Schistosoma mansoni* proteins released during in vitro miracidium-to-sporocyst transformation. Mol Biochem Parasitol 164:32–44

Yoshino TP, Bayne CJ (1983) Mimicry of snail host antigens by miracidia and primary sporocysts of *Schistosoma mansoni*. Parasite Immunol 5:317–328

Yoshino TP, Boswell CA (1986) Antigen sharing between larval trematodes and their snail hosts: how real a phenomenon in immune evasion? In: Lackie AM (ed) Immune mechanisms in invertebrate vectors. Clarendon, Oxford, pp 221–238

Yoshino TP, Cheng TC (1978) Snail host-like antigens associated with the surface membranes of *Schistosoma mansoni* miracidia. J Parasitol 64:752–754

Yoshino TP, Granath WO (1985) Surface antigens of *Biomphalaria glabrata* (Gastropoda) hemocytes: functional heterogeneity in cell subpopulations recognized by a monoclonal antibody. J Invertebr Pathol 45:174–186

Yoshino TP, Laursen JR (1995) Production of *Schistosoma mansoni* daughter sporocysts from mother sporocysts maintained in synxenic culture with *Biomphalaria glabrata* embryonic Bge cells. J Parasitol 81:714–722

Yoshino TP, Vasta GR (1996) Parasite-invertebrate host immune interactions. In: Cooper EL (ed) Invertebrate immune responses: cell activities and the environment. Springer, Berlin, pp 125–167

Yoshino TP, Cheng TC, Renwrantz LR (1977) Lectin and human blood group determinants of *Schistosoma mansoni*: Alteration following in vitro transformation of miracidium to mother sporocyst. J Parasitol 63:818–824

Yoshino TP, Wu XJ, Liu HD (1998) Transfection and heat-inducible expression of molluscan promoter-luciferase reporter gene constructs in the *Biomphalaria glabrata* embryonic snail cell line. Am J Trop Med Hyg 59:414–420

Yoshino TP, Coustau C, Modat S, Castillo MG (1999) The *Biomphalaria glabrata* embryonic BGE molluscan cell line: establishment of an in vitro cellular model for the study of snail host-parasite interactions. Malacologia 41:331–343

Yoshino TP, Boyle JP, Humphries JE (2001) Receptor-ligand interactions and cellular signalling at the host-parasite interface. Parasitology 123:S143–S157

Yoshino TP, Dinguirard N, Kunert J (2008) Gene cloning and functional characterization of a tandem-repeat galectin from the freshwater snail *Biomphalaria glabrata*, intermediate host of the human blood fluke *Schistosoma mansoni*. Gene 411:46–58

Yoshino TP, Dinguirard N, Mourão MM (2010) In vitro manipulation of gene expression in larval Schistosoma: a model for postgenomic approaches in Trematoda. Parasitology 137:463–483

Zahoor Z, Davies AJ, Kirk RS, Rollinson D, Walker AJ (2008) Disruption of ERK signalling in *Biomphalaria glabrata* defense cells by *Schistosoma mansoni*: implications for parasite survival in the snail host. Dev Comp Immunol 32:1561–1571

Zahoor Z, Davies AJ, Kirk RS, Rollinson D, Walker AJ (2009) Nitric oxide production by *Biomphalaria glabrata* haemocytes: effects of *Schistosoma mansoni* ESPs and regulation through the extracellular signal-regulated kinase pathway. Parasit Vectors 2:18. doi: 10.1186/1756-3305-2-18

Zelck UE, Von Janowsky B (2004) Antioxidant enzymes in intramolluscan *Schistosoma mansoni* and ROS-induced changes in expression. Parasitology 128:493–501

Zelck UE, Gege BE, Schmid S (2007) Specific inhibitors of mitogen-activated protein kinase and PI3-K pathways impair immune responses by hemocytes of trematode intermediate host snails. Dev Comp Immunol 31:321–331

Zhang SM, Adema CM, Kepler TB, Loker ES (2004) Diversification of Ig superfamily genes in an invertebrate. Science 305:251–254

Zhang SM, Jiang YG, Loker ES (2005) Establishing RNAi to knockdown FREP2 expression in the snail, *Biomphalaria glabrata*, an intermediate host for *Schistosoma mansoni*. Am J Trop Med Hyg 73:20

Zhang SM, Zeng Y, Loker ES (2007) Characterization of immune genes from the schistosome host snail *Biomphalaria glabrata* that encode peptidoglycan recognition proteins and gram-negative bacteria binding protein. Immunogenetics 59:883–898

Zhang SM, Zeng Y, Loker ES (2008) Expression profiling and binding properties of fibrinogen-related proteins FREPs, plasma proteins from the schistosome snail host *Biomphalaria glabrata*. Innate Immun 14:175–189

Zhang H, Wang LL, Song LS, Song XY, Wang B, Mu Ch, Zhang Y (2009) A fibrinogen-related protein from bay scallop *Argopecten irradians* involved in innate immunity as pattern recognition receptor. Fish Shellfish Immunol 26:56–64