

## Review Article

## Immunology of human schistosomiasis

D. G. COLLEY<sup>1</sup> & W. E. SECOR<sup>2</sup><sup>1</sup>Department of Microbiology, Center for Tropical and Emerging Global Disease, The University of Georgia, Athens, GA, USA, <sup>2</sup>Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

## SUMMARY

There is a wealth of immunologic studies that have been carried out in experimental and human schistosomiasis that can be classified into three main areas: immunopathogenesis, resistance to reinfection and diagnostics. It is clear that the bulk of, if not all, morbidity due to human schistosomiasis results from immune-response-based inflammation against eggs lodged in the body, either as regulated chronic inflammation or resulting in fibrotic lesions. However, the exact nature of these responses, the antigens to which they are mounted and the mechanisms of the critical regulatory responses are still being sorted out. It is also becoming apparent that protective immunity against schistosomula as they develop into adult worms develops slowly and is hastened by the dying of adult worms, either naturally or when they are killed by praziquantel. However, as with anti-egg responses, the responsible immune mechanisms and inducing antigens are not clearly established, nor are any potential regulatory responses known. Finally, a wide variety of immune markers, both cellular and humoral, can be used to demonstrate exposure to schistosomes, and immunologic measurement of schistosome antigens can be used to detect, and thus diagnose, active infections. All three areas contribute to the public health response to human schistosome infections.

**Keywords** diagnosis, immune response, immunoregulation, pathology, resistance to reinfection, schistosomiasis

## INTRODUCTION TO SCHISTOSOMIASIS

Schistosomiasis, or bilharzia, is a disease caused by trematodes of the genus *Schistosoma* (1) that afflicts at least 243 million people (2, 3). Adult male and female worms mate and produce fertilized eggs in veins of their human hosts, where they live for an average of between 3–10 years, with longevity records extending for several decades (4, 5). The eggs are excreted into the environment either in faeces or urine or are retained within the host where they induce inflammation and then die. Eggs that reach fresh water hatch and release free-living ciliated miracidia, which, if they infect a suitable snail host then reproduce asexually through mother and daughter sporocysts, producing thousands of cercariae which are released into the water and are infectious for humans. Cercariae penetrate through the skin and over 5–7 weeks migrate and mature to egg-producing adult male or female worms. Mature eggs, whether excreted or retained in the body, only live for 1–2 weeks. People can be infected by three main species of schistosomes: *Schistosoma haematobium*, *S. mansoni* and *S. japonicum*. Each species has a restricted range of appropriate snail hosts, so their transmission distribution is defined by their host snails habitat range. Adult worms live within either the perivesicular (*S. haematobium*) or mesenteric (*S. mansoni*, *S. japonicum*) venules. Schistosomes cannot excrete waste products, but rather regurgitate them into the blood stream. Some of the vomitus products are antigenic and are the basis of diagnostic assays (see below).

In areas endemic for schistosomiasis, in the absence of intervention, it is primarily a chronic disease lasting decades. This results from people being repeatedly exposed to cercariae and the longevity of adult worms. In these areas, a child's first infection often occurs by age two or three with the burden of infection increasing during the next 10 years as new worms successfully colonize the child's blood vessels (6, 7). Typical age prevalence and age intensity curves from all endemic areas show the highest

Correspondence: Daniel G. Colley, Center for Tropical and Emerging Global Disease, The University of Georgia, 500 D.W. Brooks Drive, Room 330B, Coverdell Center, Athens, GA, USA (e-mail: dcolley@uga.edu).

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prevalence, and intensities of infection are found in young adolescents. After that, they either level off or more commonly decrease to much lower levels in adults. Transmission patterns in highly endemic areas commonly show that 60–80% of school-age children are infected, while 20–40% of adults remain actively infected (8–10).

## IMMUNOLOGICAL ASPECTS OF SCHISTOSOMIASIS

The immune systems of infected hosts have several life cycle stages of the parasite that it must confront: penetrating cercariae, migrating schistosomula, adult worms and the eggs produced by adult worm pairs. All of these stages express hundreds, if not thousands, of antigenic moieties (11–13), many of which stimulate strong and easily detected humoral and cellular immune responses. Some of these responses continue to increase during chronic infection, and others are strongly down-regulated (14–17). Three main topics emerge when looking at human immune responses during schistosomiasis. (i) The most straight forward concerns immunodiagnosics, that is, what immune responses are mounted that can be used to determine whether someone has been exposed to schistosomes or if they have schistosomiasis? Many hundreds of immunodiagnostic assays have been reported and some of the more recent findings will be discussed below. (ii) Another area of immunology in schistosomiasis is that of resistance to (re)infection and responses against extant schistosomes. (iii) The third main aspect concerns immunopathogenesis and its immunoregulation. This area focuses on responses to eggs that are either exiting the body via the excreta or are trapped in bodily tissues such as the liver or bladder/urogenital wall. It is important to understand that because of the endemic and chronic nature of schistosomiasis, all three of these areas of immunologic research involve either repeated or continuous exposure to schistosome antigens over many years, thus implying ongoing changes due to antigenic exposures and the maturation of the immune responses to different levels of exposure to different antigens.

## BACKGROUND BASED ON EXPERIMENTAL STUDIES

Much of our understanding of the human immune responses to schistosomes has been facilitated by the availability of murine experimental infection models. In particular, infection of mice with *S. mansoni* exhibits many of the characteristics of human infection and has helped frame immunologic studies in people with schistosomiasis. In contrast, experimental *S. haematobium* infections have

been less instructive as adult worms do not migrate to the venous plexus and deposit eggs in the bladders of mice. However, the recent development of an *S. haematobium* egg injection model (18) has begun to yield insights into the pathogenesis of this infection (19). *S. japonicum* readily infects mice, but the challenges of working with the *Oncomelania* intermediate host have historically resulted in relatively fewer laboratories studying this species in experimental models than those working with *S. mansoni*.

Initially, the host must contend with penetrating cercariae in the skin and the subsequent larval stage, the schistosomula, as they migrate through the lungs, ending up in the mesenteric or perivesicular veins as adults. This migratory path and responses against migrating larvae and immature worms have been studied extensively in mice with the conclusion that most effective responses against incoming parasites occur in the lungs (20). Nothing is known about parasite migrations in humans, but they are assumed to be similar and they end up with the same result, adult worm pairs in specified locations. Adult worms, residing in those preferred venous environments, appear to be impervious to immune attack. Multiple mechanisms are likely to be responsible for their long-term survival in what amounts to a hostile (but impotent) immune environment. Some of these may be due, in part, in the schistosomes ability to continually regenerate its outer tegument through unique somatic stem cells (21), and perhaps their ability to masquerade through molecular mimicry (22) or by acquiring host antigens (23, 24). Some aspects of their survival may also involve manipulations of and by the hosts immune responses, such as isotypic shifts in antibody specificities (25, 26) and immunoregulation. Effective chemotherapeutic treatment of schistosomiasis does, however, depend on having established immune mechanisms that can kill the worms if they have undergone sufficient surface damage due to praziquantel (PZQ), the primary drug used to treat schistosomiasis (27–29).

Mouse models have been used extensively to investigate the protective immune response to schistosome infections, primarily with *S. mansoni* as the infecting species. Both antibodies and T cells are needed for maximal protection (30). The highest levels of protection are afforded by exposure to attenuated cercariae that die before maturity. A single exposure to attenuated cercariae induces partial protection, primarily associated with production of IFN- $\gamma$ , while antibody responses become important in the protection of animals multiply exposed attenuated parasites (31). Attenuated cercarial vaccination is effective against invading larval parasites, but their susceptibility to immune attack wanes as worms mature and become adults. Whether the mechanism is a cytotoxic attack or simply

death by delayed migration through the lungs has been questioned. Treatment of infected mice with praziquantel confers similar levels of resistance to reinfection if animals are also treated with anti-IL-10 receptor antibodies during treatment, suggesting that IL-10 ameliorates development of protective mechanisms (32).

Success with vaccination using attenuated cercariae in experimental animals led to attempts to identify individual vaccine antigen candidates based on reactivity of cells or sera from vaccinated mice. Both recombinant-protein- and DNA-based vaccines generate responses that can be enhanced by co-administration with cytokines or adjuvants that promote a Th1-type immune profile. Unfortunately, these vaccines have been less effective and less reproducible than immunization using attenuated cercariae. Nevertheless, this work provided the basis for the two schistosomiasis vaccine candidates currently being tested in humans (33, 34). Second generation vaccine candidates have focused more on generating immune responses to molecules that play a functional role in parasite homeostasis, such as membrane turnover, nutrient uptake or neutralization of reactive oxygen species (35–38). Vaccines in the latter category may even have therapeutic activity by disrupting adult worm immune evasion mechanisms and rendering them susceptible to host effector mechanisms.

The eggs produced by adult worms in their venous locations are intended (from the worms perspective) to be carried out of the body either by faeces or urine and released into the environment. However, the venous blood flow carries many of the eggs in the opposite direction or prevents their easy escape. The eggs contain a wide variety of proteases and other potentially toxic moieties, which once they are lodged in the tissues, can lead to necrosis (39, 40). The hosts defence against this tissue insult comes in the form of granuloma formation, to wall off and contain the egg and the proteolytic products it releases. The granulomas themselves can be detrimental lesions, and to prevent them from overwhelming the tissue sites or blocking venous blood flow, immunomodulation of the anti-egg antigen responses (granuloma formation) develops effectively in mice (41) and most people upon the establishment of chronic infections (42–44).

Key roles for the immune response in worm maturation and granuloma formation have been demonstrated through experimental *S. mansoni* infections of T-cell-deficient mice (40, 45). During the initial stages of infection, mice display a balanced or Th1-type immune response to parasite antigens. However, once egg deposition begins around 6 weeks of infection, a dramatic shift to a Th2-type response ensues. Specific schistosome egg antigens interacting with dendritic cells are responsible for this immunologic shift,

partially through the action of certain carbohydrate epitopes (46). Unregulated production of the Th2 cytokine IL-13 eventually leads to widespread liver fibrosis, the functional cause of hepatosplenic disease in humans (47, 48). However, depletion of Th2 responses, particularly IL-4, results in tissue damage and host mortality due to pro-inflammatory Th1-type responses (49, 50). Thus, Th2 responses also perform a host protective function, and appropriate regulation minimizes overall host pathology. Alternatively activated macrophages and IL-10 are part of the regulatory feedback of Th2-type responses that limit the initial granulomatous inflammation that peaks in size and intensity at 8 weeks of *S. mansoni* infection (51, 52). As the infection continues, these and other immunomodulatory mechanisms further regulate granuloma formation such that newly deposited eggs at 12 or more weeks of infection induce smaller granulomas and less fibrosis than during the acute stage (53, 54). Failure to modulate the granulomatous response results in a hypersplenomegaly syndrome that shares many pathologic and immunologic characteristics with human hepatosplenic disease, greater fibrosis and shunting of worms and eggs to the lungs (55, 56). Mechanisms of immunomodulation include IL-10, T regulatory cells, B cells, antibodies, anti-idiotypic responses and T cell anergy (57, 58).

Egg excretion from mice is also dependent on the immune response, with T-cell-deficient mice demonstrating fewer faecal eggs (59). Recently, a predilection for schistosome egg deposition in Peyer's Patches, which stimulated vascular remodelling and egg excretion, has been demonstrated (60).

## HUMAN IMMUNE RESPONSES DURING SCHISTOSOMIASIS

When studying or reading about human immune responses during schistosomiasis, it is critical to consider the multiple facets of the host–parasite interface described above that involve different parasite life cycle stages. These are important distinctions for the immunologist, because they are important discriminations made by the hosts immune responses. Regardless of the endemic area in which studies are carried out, there is an overriding differential pattern of immune responses against worm-derived antigens vs. egg-derived antigens (61). In most studies, this is seen as early high-level responses to soluble egg antigens (SEA) that then decrease as infections become chronic (42–44, 62–64). Responses to soluble worm antigenic preparations (SWAP), in contrast, invariably rise during early infection and continue to be expressed throughout continuing chronic infections. This has long been true using these crude antigenic mixtures and is being shown now to be

true for individual antigenic moieties expressed by different life cycle stages (65). It is also important to distinguish the status of people being studied beyond just whether or not they are currently harbouring schistosomes by considering how long they have been infected (66), whether their mother was infected while they were *in utero* (67, 68), and whether and how often they may have been treated for their schistosomiasis with PZQ (69–72). All of these situations and probably many others contribute to their immune status at the time they are being studied.

In addition, it should always be remembered that the study of human immune responses in schistosomiasis is almost exclusively based on the preformed circulating antibodies or cytokines or the responsiveness of lymphoid cells in the peripheral blood. These sources may or may not be representative of what is occurring in the micro-immunoenvironment of either the granulomatous lesion or against incoming schistosomes. Nevertheless, these are the specimens available to investigators, except in rare instances when spleens or other tissues can be obtained either at surgery or autopsy. Regardless of these stipulations, multiple investigators have successfully defined many aspects of the human humoral and cellular immune responses to schistosome antigens in relation to pathology, resistance to reinfection and diagnostics.

#### IMMUNOLOGY OF MORBIDITY AND REGULATION OF MORBIDITY IN HUMANS

As in experimental animal models, morbidity during human schistosomiasis results from chronic immune stimulation by schistosome eggs that are trapped in tissues and subsequent granuloma formation and fibrosis (73, 74). The vast majority of the burden of disease due to *S. mansoni* and *S. japonicum*, and possibly *S. haematobium*, appears to be caused by chronic inflammation, resulting in subtle morbidities such as anaemia, growth deficiencies, physical fatigue and diminished cognitive development (75–79). The inciting insults of this chronic inflammation are soluble egg antigens released from tissue-trapped eggs (80). While normal liver enzyme patterns are generally maintained during chronic schistosomiasis unless severe pathology develops (81), indicators such as increased levels of hepcidin imply that inflammatory processes are at the heart of subtle morbidity due to these granulomatous lesions (76, 82, 83). In *S. haematobium* infections, the anaemia of chronic inflammation is aggravated by the blood loss seen as gross and microhaematuria. Along with these examples of direct morbidity, schistosome infections can have indirect effects such as predisposing infected hosts to greater susceptibility to other pathogens. For example, the friable sandy patches

seen in female genital schistosomiasis caused by *S. haematobium* infections are associated with an increased risk of HIV acquisition (84, 85).

The immune process of granuloma formation, left unimpeded, would soon occupy vast amounts of tissue space, eventually shutting down return blood flow back to the heart through the portal system, creating portal hypertension, pulmonary hypertension and ultimately oesophageal varices, resulting in death. Prior to regular treatment with praziquantel of children and adults in high-risk occupations, this picture was seen in proportions of those infected with either *S. mansoni* or *S. japonicum* varying from 2 to 25% (86). The fact that it did not occur more frequently is in part attributable to immunomodulation of responses to SEA, as reflected in reduced lymphocyte proliferation in patients that do not develop hepatosplenomegaly (44). This phenomenon has been examined in human schistosomiasis by multiple groups, resulting in the consensus hypothesis that continuous exposure to SEA leads to the induction of regulatory mechanisms that dampen down granuloma formation, anti-IgE antibody production, and SEA-induced lymphocyte proliferation and cytokine production (44, 62, 87–89). A number of immunoregulatory mechanisms have been identified through investigations using cells and antibodies from chronically infected intestinal patients with subtle morbidity. These include adherent, macrophage-like cells (90); immune complexes (91); IL-10 (92); TGF- $\beta$  (93); T regulatory cells (16, 71); and idiotypic interactions (94). It is impossible to ascribe an attributable fraction to each of these mechanisms, because they are demonstrated *in vitro* and with only correlations to states of morbidity. However, taken in the aggregate, and in the face of repeated findings by multiple groups in multiple endemic areas, down-regulation of SEA responses is occurring during chronic schistosomiasis and contributes to the establishment and ability to maintain chronic infections over decades without the development of hepatosplenomegaly by most infected individuals (44, 86). In addition, immunogenetics contributes to the ability of some people to better regulate (or not) their immune responses to schistosome infections (95, 96).

The concept that active schistosomiasis during pregnancy might impart an altered immune status on the offspring has been studied over a long span of time (58, 97, 98). There is evidence that this form of immune manipulation *in utero* actually occurs in humans because newborns of mothers with schistosomiasis already express IgM or and IgE antischistosome antibodies and have increased percentages of mature, CD5- B cells in their cord blood (68, 99). Also, it has long been known that their cord blood mononuclear cells proliferate strongly in response to SEA (but not *Trypanosoma cruzi* antigens,

unless the mother also has Chagas disease) and idiotypes on anti-SEA antibodies (67). In regard to the specificity of these responses, the cord blood mononuclear cells of babies born to mothers who have Chagas disease respond to *T. cruzi* antigens, but not to SEA (67). While this antigen and idiotype sensitization occurs *in utero*, as do other influences on B cells (99), the impact of such perinatal influence is not known. It is hypothesized that it may result in an early immunoregulation against SEA, allowing the majority of children in an endemic area to establish regulated, chronic infections (58, 100). Other perinatal influences are noted in the article by Dr Alison Elliott in this issue.

It should be remembered that each of these interesting findings needs to be substantiated in various patient populations, and eventually mechanistic studies should be pursued to establish how the various phenomena fit together to provide the appropriately regulated responses that allow both host and parasite to survive. It is essential that individual findings be validated or compared based on different patient populations, exposure patterns, stage of infection, durations of infection and the like. It is hoped that reviewers and editors understand this necessity of having confirmatory evidence of any given finding or mechanism in such a complex relationship. The publishing of the first finding of high proportions of Treg in patients with schistosomiasis mansoni serves as an example (71). While interesting, it is critical that such findings be repeated both in the same populations and in other populations (16). Once substantiated, such observations need to then be studied functionally in well-characterized subjects with different clinical forms or durations of infection as well as in other endemic settings. New cell subtypes are being defined almost constantly, diminishing the likelihood that pathology will be easily attributable to a given cell producing a given mediator in response to a given antigen. Even whether the most relevant antigens are secreted, located in membranes, or are somatic remains a topic of debate. Perhaps recent advances in schistosome proteomics will facilitate better definition of the critical antigens for human immunopathology.

## IMMUNOLOGY OF RESISTANCE TO REINFECTION IN HUMANS

Whether a protective resistance to reinfection exists in people has long been discussed (101), but several lines of evidence now indicate that it does develop, although it may take a long time (17, 102) and perhaps rarely results in sterile immunity. A number of studies suggest that worm death, occurring either naturally or upon treatment, leads to the release of immunogens that stimulate

protective responses, which after a sufficient number of occurrences are at a level to effectively react with antigens expressed by susceptible incoming schistosomula (17, 25, 69, 70, 72, 103–106) or lead to a decrease in fecundity (107). Despite the challenges of evaluating reinfection rates in people who have different exposure histories, encounter water bodies with differences in force of transmission and can be quite distinct genetically, epidemiologic data in endemic populations generally support age-associated decreases in infection as a result of development of anti-parasite immunity, as opposed to reduced water contact (108). However, while children in endemic areas are usually more susceptible to infection and reinfection than adults, this may not be entirely linked to histories of exposure and infection (15, 109). Part of developing an understanding of resistance to infection or reinfection, and how treatment may promote it, involves identifying immune responses that correlate with protection.

Unlike mice that demonstrate resistance in association with Th1-like responses, human immune responses that have repeatedly been linked with resistance to schistosomiasis reinfection are more Th2-associated. The association between parasite-specific IgE, eosinophils and resistance to reinfection has been observed across infecting schistosome species and in a variety of epidemiologic settings (26, 110–115). Mechanistically, both high- and low-affinity IgE receptors on eosinophils and B cells (or in soluble form), respectively, are associated with protection against reinfection (70, 116, 117). In contrast, susceptibility to reinfection has been associated with IgG4, which may serve as a blocking antibody, inhibiting the action of IgE (111–115, 118). Interestingly, the propensity of children and adults to produce IgG4 and IgE, respectively, matches their relative susceptibility to reinfection (119). Following treatment of adults, adult worm-specific IgG4 levels decrease, while worm-specific IgE is maintained at pretreatment levels or increases. In children, who more readily become reinfected, treatment is less likely to increase the IgE/IgG4 ratio. Recently, certain *S. mansoni* adult worm-associated tegumental-allergen-like (TAL) proteins have been characterized as important potential targets of protective IgE and reinfection-associated IgG4 (25, 120, 121).

Cytokine responses to schistosome antigens are also altered by treatment. IL-4 and IL-5, cytokines associated with stimulation of IgE and eosinophil production, respectively, generally increased following praziquantel treatment (122–127). Resistance to reinfection has been associated with these responses to the tegument antigen paramyosin in persons infected with *S. japonicum*, and soluble adult worm antigen preparations in persons infected with *S. haematobium* (14, 128). IFN- $\gamma$  production following treatment is more commonly (although not exclusively)

linked with susceptibility to reinfection, as is IL-10 (129, 130). Interestingly, IL-10 is associated with IgG4 production (131), consistent with the observations that IgG4 responses are associated with susceptibility and the findings in mice that blocking IL-10 receptors is necessary for treatment to induce protection against reinfection (32). As with human immune correlates of immunopathogenesis and immunoregulation, critical antigens and immune cell correlates of resistance to reinfection need confirmation between different populations in a variety of epidemiologic situations to substantiate the relevance of any given response in host protection.

#### STATUS OF VACCINE CANDIDATES AND THE POTENTIAL ROLE IN ELIMINATION

There have been two schistosome candidate vaccines that have been produced by good manufacturing procedure and entered Phase I safety and immunogenicity trials – ShGST (Bihvax) (33) and Sm14 (34). The results of the Bihvax Phase I trial have been published, but those from the Sm14 trial are being analysed at this time. Bihvax has also undergone Phase II and III trials in West Africa, and those results are also currently being analysed. Thus far, these candidates have not induced adverse reactions, but have induced immune responses. Other candidates that are in preclinical trials at various stages include tetraspanin-2 (132) and calpain (133) for *S. mansoni* and paramyosin (134), triose-phosphate isomerase (135), tetraspanin (136) and schistosome insulin receptor (137) for *S. japonicum*. Many of the vaccines for *S. japonicum* are being developed to help control the transmission contribution of zoonotic host species.

There are many questions that need to be addressed as research on schistosome candidate vaccines move forward (138). The first, and perhaps most contentious, question is, Do we need such a vaccine to control schistosomiasis? The debate has been further fuelled by the World Health Assembly Resolution 65.21 call to eliminate schistosomiasis. Perhaps the best answer to that is twofold: first, if we had an effective vaccine, we would use it. It is clear that in most endemic countries, mass drug administration with PZQ will not be sufficient to eliminate schistosomiasis; therefore, we need new tools to be used in combination with MDA such as snail control, behavioural change, water and sanitation. The second question might be, What is the ideal Target Product Profile is for a vaccine to protect against acquisition of schistosomiasis, or in fact could it be a vaccine that would simply reduce morbidity through control of egg production? In addition to the fundamental questions about the need or type of vaccine, there are other questions regarding how a vaccine should

or could be safely tested. Even once additional antigens are ready for human testing, the design of clinical trials to evaluate them will produce its own challenges (138). Current experimental schistosomiasis vaccine candidates are evaluated by their ability to reduce worm burdens upon challenge infection and perfusion, but this approach cannot be used in humans because it is currently impossible to quantify human schistosome worm burdens. Egg output is another possible measure of vaccine efficacy, but we do not know the true correlation between quantitative egg output and worm burdens, and furthermore, it is likely not stable throughout infection. Challenge infections are not acceptable in a situation where adverse events such as transverse myelitis could occur prior to evaluation by egg output. Similarly, testing a vaccine on large populations of people in endemic areas when they could also be treated with PZQ might be ethically challenging and contentious. This is all ignoring the substantial cost investment needed to get through clinical testing and regulatory requirements. Nevertheless, the potential long-term role that an appropriate vaccine could play in the elimination of schistosomiasis, and the sustaining of that task makes continued studies on the discovery and development of antischistosome vaccines a worthy goal.

#### IMMUNODIAGNOSTICS

The accepted diagnostic standard of schistosomiasis is evidence of viable eggs in urine (*S. haematobium*), faeces (*S. japonicum*, *S. mansoni*) or tissue biopsies. These microscope-based assays are relatively insensitive, especially in situations involving low level infections (139, 140). Molecular techniques for schistosome DNA detection in faecal, urine or blood specimens increase sensitivity, but are expensive and still suffer somewhat from sampling limitations (141, 142). Serologic assays have proven useful clinically (143) for diagnosis by the detection of antibodies against schistosomal antigens. This approach, with an extremely wide variety of reported immunodiagnostic assays, is particularly useful for symptomatic travellers or for serosurveys. However, for people in areas endemic for schistosomiasis, current serologic tests cannot discriminate between active infection and past exposure, although some isotypic assays can generally group active or inactive infections (119, 144, 145). Circulating schistosomal antigen detection by monoclonal antibodies has been reported for decades and has the advantage of detecting active infections in a semi-quantitative manner. There is now a point-of-contact circulating cathodic antigen (POC-CCA) assay commercially available for mapping of *S. mansoni* infections. This lateral flow cassette assay is performed on urine (Rapid Medical Diagnostics, Pretoria, RSA) and

appears more sensitive than the Kato-Katz assay for mapping of *S. mansoni*-endemic areas (140), allowing on-site mapping of *S. mansoni* without stool collections. This will provide an important tool for introduction of control programmes into new areas. However, more sensitive and specific immunodiagnostic tools will be needed for field studies, vaccine and drug testing, elimination programmes, and in actual clinical diagnostics. Again, these efforts may be assisted by proteomic and metabolomic studies that may identify specific antigens or biomarkers for sensitive infection detection. Recent development of PCR diagnostic techniques are a welcome addition, but these assays still suffer from a sampling limitations of urine or stool, whereas a more useful diagnostic would utilize serum or dried blood spots that could be multiplexed for assays for other infections. It also bears remembering that none of the literature or assays available provides an actual number of worms with which someone is infected. We are, instead, left with correlates of worm burden that are at best estimates and have little or no basis in data from people with active infections at different times after the establishment of their infections. This lack of a true gold standard is an impediment to many activities and studies on human schistosomiasis.

#### WHAT IS LEFT?

There is obviously much more information needed to truly understand the complex relationships between schistosomes and their human hosts. When discussing these interactions, it is important to keep in mind whether the topic is immunity against infection/reinfection or immunopathogenesis and to realize that there are almost certainly multiple responses and regulatory responses that play off against each other in support of a semi-balanced chronic infection. It is also useful to admit that the approaches and tools we have are not ideal. We are essentially restricted to the use of peripheral blood as our window into immune responses that are undoubtedly actually being played out in tissue microenvironments. Furthermore, without the option to infect and treat or to use manipulated parasites in controlled studies, we are confined to correlations rather

than proofs. Finally, movement of *S. mansoni* into areas traditionally dominated by *S. haematobium* and of *S. haematobium* into areas endemic for *S. mansoni* complicates the epidemiologic, immunologic and diagnostic picture, especially with respect to the recent description of interspecies hybridizations between different human and animal schistosomes, which may alter the host–parasite interactions in both the mammalian and molluscan hosts (146, 147).

Still, there are many questions to be answered and careful correlative studies between various immune responses and well-documented cases or treated individuals will yield critical answers. Some of the immunologic questions that remain are obvious: What are the actual mechanisms of killing of invading schistosome? Are there regulatory responses that hinder these mechanisms? How do adult schistosomes survive in an immunologically active environment? What level of granuloma formation is required to thwart the damaging effects of egg constituents and how much granuloma formation is too much, resulting anaemia of chronic inflammation or severe disease? What combination of regulatory mechanisms determines whether the outcome is anaemia or hepatosplenic or urinary fibrosis? Do the antischistosome immune responses induced by being born of an infected mother establish regulatory or protective status? Does having schistosomiasis effectively down-regulate someone's ability to respond appropriately unrelated immunizations or co-infections? Does having schistosomiasis effectively down-regulate someone's ability to respond inappropriately to other stimuli, such as allergens or auto-immunogens? Appropriate studies of people with schistosomiasis are yielding and will continue to yield answers to these critical questions.

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