

# Chapter 3

## Schistosomiasis

Ahmad Othman and Rashika El Ridi

**Abstract** Schistosomiasis is one of the most imposing and widespread helminthic diseases. The immune system strives to be protective vis-à-vis *Schistosoma* infection, but the inevitable immunopathology may lead to fibrosis and organ dysfunction. Moreover, the skewing of immune response axis to polarized Th2 phenotype can impair resistance to other pathogens and has been associated with neoplasms, further complicating the clinical sequelae of schistosomiasis. Techniques and tools for diagnosing schistosomiasis are either cumbersome or lack sensitivity and specificity. Accordingly, many patients remain undiagnosed and receive no treatment. Currently, the only available drug is praziquantel; the use of which in mass treatment raises concerns about development of drug resistance. Indeed, *Schistosoma* infection is a fascinating model for gaining insight about the mutual interplay between host and parasite factors, which ultimately determines the overall morbidity. Despite decades of intensive research on schistosomiasis, unresolved issues are still intriguing scientists, one of which is the development of a vaccine. Fortunately, however, significant progress has been achieved towards the elimination of schistosomiasis. Information provided in this review should help opening venues for better diagnosis, treatment, and prevention of schistosomiasis.

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A. Othman (✉)  
Medical Parasitology Department, Faculty of Medicine, Tanta University, Tanta,  
Gharbiya, Egypt  
e-mail: [ahmed\\_ali44@hotmail.com](mailto:ahmed_ali44@hotmail.com)

R. El Ridi  
Zoology Department, Faculty of Science, Cairo University, Cairo 12613, Egypt

### 3.1 Introduction

Millions of children suffer anaemia, growth deficiency, abdominal pain, exercise intolerance, poor school performance, cognitive defects, and other sequelae resulting from infection with schistosomes. Millions of adult males and females endure fever, headache, lethargy, and lowered work capacity and quality of life because of severe lesions and damage in the liver, colon, rectum, and/or bladder and lower urinary tract consequent to the infection. At least 200,000 people die annually of haematemesis, liver failure, or cancer of the urinary bladder. Sound information is required for setting the platform for elimination of this serious affliction.

### 3.2 The Agent

Schistosomes are flatworms (kingdom Animalia, phylum Platyhelminthes, class Trematoda), which are exclusively different from other trematodes in having separate sexes; yet, they form pairs, mimicking the hermaphrodite condition. They are endoparasites (subclass Digenea) of an intermediate host snail where they reproduce asexually and a final vertebrate host where copulation of adult male and female leads to daily production of hundreds of eggs. They belong to the order Strigeidida, characterized by a forktailed cercaria, which infects final hosts using enzymes of penetration glands. The suborder is Strigeata, superfamily Schistosomatoidea, which contains three families: Sanguinocolidae (parasites of fish), Spirorchidae (parasites of turtles), and Schistosomatidae (parasites of crocodilians, birds, and mammals). They demonstrate unsurpassed precision in laying eggs near the conduit for egg passage to the external environment to complete the life cycle. The family Schistosomatidae comprises approximately 100 species, among which *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum* and to a less prevalent extent *S. guineensis*, *S. intercalatum*, and *S. mekongi* cause human schistosomiasis, also named bilharzia, a severe disease of humans in 74 developing countries of the tropics and subtropics (Platt and Brooks 1997).

### 3.3 Epidemiology of Schistosomiasis

Incidence and prevalence of schistosomiasis reflect the distribution of the freshwater snail the schistosome species use as intermediate host. Thus, the presence of *Oncomelania* snails (*Oncomelania hupensis*) in the Philippines (Leonardo et al. 2013) and the marsh and lake regions of southern China (Li et al. 2000; Ross et al. 2001; Zhao et al. 2012a, b) allows the spread of *S. japonicum*. In the Middle East and sub-Saharan Africa, *Oncomelania* spp. and schistosomiasis *japonicum* are not found, while the broad geographical range of susceptible snail

species of the genus *Biomphalaria* and *Bulinus* coincides with the widespread incidence of schistosomiasis mansoni and schistosomiasis haematobium, respectively (Morgan et al. 2001). In contrast, schistosomiasis is absent from Cape Verde, Comoros, and Seychelles owing to the absence of permissive snail intermediate hosts (Utzinger et al. 2009). In Brazil, the distribution of *Biomphalaria* spp. (*B. glabrata*, *B. straminea*, *B. tenagophila*) is closely associated with the occurrence of schistosomiasis mansoni (Scholte et al. 2012). Transmission of the parasite to the human population in Zanzibar is related to the distribution of the intermediate snail host, *Bulinus globosus* (Allan et al. 2013). Abundance of *Bulinus truncatus* is declining in Egypt and so is urinary schistosomiasis (Barakat 2013). On the other hand, the occurrence of *B. truncatus* and *B. beccari* in Saudi Arabia might allow spread of *S. haematobium* (Mostafa et al. 2009, 2012).

The prevalence of schistosomiasis is also closely related to adequate financial, social, and political conditions that would permit rise in living standards and access to clear water, improved sanitary conditions, and health education to every resident of rural areas (Utzinger et al. 2009, 2011; King 2010). Thus, schistosomiasis transmission was reported to be interrupted in Japan, Iran, Jordan, Morocco, and Tunisia (Utzinger et al. 2009, 2011; World Health Organization 2011). In contrast, more than 90 % of all estimated cases of hepato-intestinal and urinary schistosomiasis reside in sub-Saharan Africa with Nigeria and Tanzania having the highest burden. Of note, the prevalence of infection increased with increase in the population in Tanzania from 19 % in 1977 to 51.5 % in 2012 (Mazigo et al. 2012). On the other hand, the zoonotic behaviour of *S. japonicum*, which may infect 40 mammalian species, renders eradication of schistosomiasis in China a most difficult task (Ross et al. 2001; Zhou et al. 2012; Hong et al. 2013).

Additionally, epidemiology of schistosomiasis is directly related to completeness and accuracy of information regarding infection prevalence. There is a prominent lack of reliable and updated information on schistosomiasis prevalence in numerous countries (Brooker et al. 2010; Utzinger et al. 2011) as the most complete global resource (Atlas of the Global Distribution of Schistosomiasis) is 26 years old (Brooker et al. 2010; World Health Organization 2010). Thus, several reports indicate that the prevalence rate of schistosomiasis in Egypt has dropped to nearly 1.5 % (EMRO 2007; Curtale et al. 2010), while no national programme for investigation of schistosomiasis prevalence was heard of since the year 2000. Thus, in the Abis area (near Alexandria), which is reportedly a region of “low” *S. mansoni* prevalence and intensity, 15.2 % of 11-year-old children were found to be infected with *S. mansoni* with infection intensity of up to 480 egg/g (Allam et al. 2009). Understandably, international travellers (not local residents) were warned that “Egypt is one of the most highly infected countries” (IAMAT 2012).

As importantly, epidemiology is dependent on the level of accuracy of diagnostic methods. Thus, the World Health Organization (WHO) estimate is of 235 million cases of schistosomiasis worldwide, with 732 million people at risk for infection in known transmission areas (WHO 2009). Since standard methods of field testing are admittedly insensitive (Gryseels 1996; Gad et al. 2011), true prevalence and worm loads in endemic communities may be considerably underestimated, and

accordingly, the WHO often-quoted statistics can only represent floor estimates for active and potential cases (de Vlas and Gryseels 1992; Enk et al. 2008; Coulibaly et al. 2012). King (2010) argued that probably 40–60 % of patients are likely misdiagnosed and hence suggested that the WHO values should be adjusted to between 391 and 587 million people worldwide. As a consequence, the WHO Preventive Chemotherapy and Transmission Control (PCT) databank no longer provides estimates on population infected or at risk. These have now been replaced by estimates of “population requiring preventive chemotherapy”, which is the population in need of regular treatment with praziquantel, based on WHO recommendations and prevalence thresholds, calculated by “implementation unit”. This indicator is now used in order to avoid the biases inherent in the calculation of number of people infected (by reason of the low sensitivity of the diagnostic tools used) or population at risk (by reason of the geographical focality of the disease) (WHO PCT databank—schistosomiasis. [http://www.who.int/neglected\\_diseases/preventive\\_chemotherapy/sch/en/index.htm](http://www.who.int/neglected_diseases/preventive_chemotherapy/sch/en/index.htm)).

*Schistosoma mansoni* is endemic in a few countries in South America, principally Brazil, where prevalence rate is less than 1 %, and in around 50 countries in the Middle East (prevalence rate in Egypt less than 10 %) and sub-Saharan Africa where prevalence rates exceed 50 % in Nigeria, Ghana, Mozambique, Burkina Faso, Mali, Sierra Leone, Madagascar, and Tanzania (Utzing et al. 2009, 2011; WHO 2010). In Tanzania, various studies have reported prevalence of up to 100 % in some areas at the Lake Victoria shores (Mazigo et al. 2012). Schistosomiasis haematobium is prevalent only in the Middle East and sub-Saharan African countries (Hürlimann et al. 2011).

Approximately 12.7 million people are infected with schistosomes in the Middle East and North Africa with 7.2 million accounted for in Egypt, 2.9 million in Yemen, 2.3 million in Algeria, and 0.3 million in Libya (Steinmann et al. 2006; Hotez et al. 2012). More than 12,000 schistosomiasis recent prevalence survey locations from 35 Western, Middle, Eastern, and Southern African sub-Saharan countries are now compiled in one database that can be accessed at <http://www.gntd.org> (Hürlimann et al. 2011). *S. haematobium* (54.4 %) and *S. mansoni* (40.8 %) were the most prevalent species. The third schistosome species parasitizing humans in Africa, *S. intercalatum* (4.8 %), was only reported in surveys carried out in Cameroon and Nigeria, confirming that this species is restricted to some parts of West and Central Africa. The co-occurrence of *S. mansoni* and *S. haematobium* was reported in 20 % of the survey locations. Based on the WHO prevalence cut-offs of <10 %, 10–50 %, and >50 % as low-, moderate-, and high-endemicity areas, respectively, *S. haematobium* is highly prevalent in Western and Southern sub-Saharan African countries, while *S. mansoni* predominates in East Africa (Hürlimann et al. 2011).

*Schistosoma japonicum* affects humans and more than 40 mammalian host species, all of which can act as reservoirs of infection, in the Philippines, China, and Indonesia. However, only 7 out of 12 previously endemic provinces in China still report schistosome infections with prevalence rates of 6–7 % (Peng et al. 2010; Zhou et al. 2011a, b, 2013). In the Philippines, there are currently only 560,000

cases of schistosomiasis (Bergquist and Tanner 2010; Gordon et al. 2012). Schistosomiasis transmission is also under control in Indonesia in the two previously endemic areas of Lindu valley and Napu valley, both located in the province of Central Sulawesi, where prevalence rates range between 0 and 13 % (Garjito et al. 2008).

### 3.4 Immunopathogenesis of *Schistosoma* Infection

Barsoum et al. (2013) stated that “almost all clinical features of schistosomiasis are caused, directly or indirectly, by the host’s immune response to different stages of the parasite’s life cycle in the body”. Most of our concepts of the immunopathological processes during *Schistosoma* infection are derived from animal studies especially on *S. mansoni* and *S. japonicum* and much less frequently on *S. haematobium*. Human studies are relatively few and difficult to be controlled and interpreted. Most authorities, however, believe that the immunopathology is rather similar in human and animal hosts.

#### 3.4.1 Acute Schistosomiasis

**Cercarial dermatitis** is a local IgE-mediated hypersensitivity response directed against penetrating cercariae. It occurs infrequently among endemic populations but is common among visitors and migrants and after primary infections (Gryseels et al. 2006). Upon adherence to the host skin, *S. mansoni* cercariae exposed to linoleic acid produce prostaglandin E2 (PGE2) and induce mouse and human keratinocytes to produce PGE2 and immunosuppressive interleukin (IL)-10 (Ramaswamy et al. 2000). After invasion of the host skin, cercariae transform into schistosomula and release large amounts of proteins that can activate lymph node cells of irradiated cercariae-vaccinated mice to release copious amounts of interferon (IFN)- $\gamma$  (Harrop et al. 2000). In vivo, cercariae-derived proteins likely interact with the Langerhans cells and keratinocyte surface membrane toll-like receptor (TLR)2 and TLR4 and/or mannose-binding lectin, leading to the production of nitric oxide, inflammatory cytokines, IL-10, and PGE2 (Ramaswamy et al. 2000; Pivarcsi et al. 2004). Additionally, upon entry in the dermis, skin-stage schistosomula secrete a *S. mansoni*-derived apoptosis-inducing factor of 23 kDa, which elicits apoptosis in the CD4+ and CD8+ T lymphocytes surrounding the larvae in the skin of naive and irradiated cercariae-vaccinated mice (Chen et al. 2002). Blood cell cultures of humans infected with *S. mansoni* and/or *S. haematobium* stimulated with cercarial excretory–secretory products (ESP) also released large amounts of IL-10 (Turner et al. 2013). Fluorescent imaging revealed uptake of cercarial and schistosomular ESP by murine skin neutrophils, dendritic cells, and macrophages, with subsequent production of IL-6, tumour

necrosis factor (TNF), IL-12, and IL-10 (Paveley et al. 2009). Indeed, skin schistosomula ESP-mediated immune responses lead to both immune priming and regulation (Mountford and Trottein 2004).

**Katayama syndrome** is a systemic immune complex-mediated hypersensitivity reaction against migrating schistosomula and early egg deposition. The symptoms of Katayama syndrome manifest 14–84 days after individuals are first exposed to schistosome infection or following heavy reinfection. Acute schistosomiasis due to *S. mansoni* or *S. haematobium* infection is common among individuals exposed for the first time such as travellers or migrants but is rare among endemic populations. In contrast, acute disease due to *S. japonicum* is common in endemic communities and is associated with severe and persistent manifestations that may rapidly progress to hepatosplenomegaly and portal hypertension (Gryseels et al. 2006; Ross et al. 2007; Burke et al. 2009).

### 3.4.2 Chronic Schistosomiasis

The course of chronic schistosomiasis is variable and is dependent on the anatomical location of adult schistosomes within the vasculature of the mammalian host. In murine models, immune responses to schistosome antigens manifest a striking shift from a moderate T-helper type 1 (Th1) to a robust Th2-dominated response. Fibrosis and much of the pathology are primarily mediated by Th2, while Th1 responses are presumed to be protective (Reiman et al. 2006). However, recent evidence suggests that maintaining a balanced and controlled Th1 or Th2 response is critical in the case of schistosomiasis for protective granuloma formation without excessive pathology (Wilson et al. 2007).

During the first 4–6 weeks of infection in the mouse, a moderate Th1 response is induced against migrating schistosomula and immature adult worms. This response exhibited increased levels of circulating proinflammatory cytokines including TNF- $\alpha$ , IL-1, IL-6, and IFN- $\gamma$  (Pearce and MacDonald 2002; Wynn et al 2004; Wilson et al 2007). High levels of these cytokines have also been associated with the development of Katayama syndrome in humans (Caldas et al. 2008). The immune response then polarizes to a Th2 response with the start of egg laying, characterized by augmented expression of IL-4, IL-5, IL-10, and IL-13. The Th2 response reaches a peak at approximately 8 weeks postinfection and is then downregulated with progression to chronic infection (Pearce and MacDonald 2002; Wynn et al. 2004; Wilson et al. 2007).

The situation is more complex in humans as the cytokine profile in chronic cases is typically a variable *mix* of Th1 and Th2 cytokines. Different clinical entities of schistosomiasis are associated with characteristic cytokine patterns (Caldas et al. 2008). For example, one study revealed that whole blood cultures of approximately 340 Egyptian schoolchildren patently infected with *S. mansoni* produced large amounts of IFN- $\gamma$  and IL-17 in response to soluble adult worm antigens (SAWA), soluble egg antigens (SEA), and recombinant schistosome antigens,

namely, glyceraldehyde 3-phosphate dehydrogenase (rSG3PDH), challenging the dogma that the immune responses to schistosome antigens are dominated by type 2 cytokines, principally IL-4 and IL-5, while Th1 responses are downregulated in human schistosomiasis. Furthermore, the blood cultures of approximately 60 % of these children produced IL-4 or IL-5 to SAWA and SEA (but not to rSG3PDH) only following praziquantel treatment (Barakat et al. [manuscript under preparation]). These findings are in accord with previous reports showing that plasma IL-5, IL-13, and IL-33; serum IgE binding to adult worm antigens; and circulating eosinophil numbers significantly increased only upon treatment in children and adult patients with patent schistosomiasis mansoni (Joseph et al. 2004b; deMoraes et al. 2008) or haematobium (Wilson et al. 2013a, b). In other studies of humans with chronic schistosomiasis mansoni, whole blood cultures of adults (Joseph et al. 2004a) and 4–17-year-old children (Wilson et al. 2008) produced significantly ( $P < 0.05$ – $<0.001$ ) higher levels of IL-4, IL-5, and IL-13 in response to SAWA than to SEA.

In sum, research has indicated that patently infected mice and praziquantel-treated humans produce large amounts of type 2 cytokines and the immunosuppressive IL-10 in response to SEA (Joseph et al. 2004a, b; Wilson et al. 2008), likely leading to the downregulation of granuloma formation and liver fibrosis. On the other hand, persistent production of low levels of IL-10 and IFN- $\gamma$  and high levels of inflammatory cytokines appears to be associated with severe periportal fibrosis, hepatosplenomegaly, and portal hypertension (Hoffmann et al. 2000; Booth et al. 2004; Wilson et al. 2008; Barsoum 2013).

#### 3.4.2.1 Granuloma Formation

The eggs of *Schistosoma* deposited in the tissues induce granuloma formation. Upon full maturation, the living embryo, the miracidium, secretes SEA which exit via the microscopic pores of the egg shell (Ashton et al. 2001). For *S. mansoni*, the egg-derived antigens include Sm-40, cytoskeletal proteins like tubulin, egg-secreted protein 15, proteins of the micro-exon gene 3 (MEG-3) family, as well as the IL-4-inducing factors of *S. mansoni* eggs, IL-4-inducing principal of *S. mansoni* eggs (IPSE), and the ribonuclease domain-containing Omega 1 (Fitzsimmons et al. 2005; Jang-Lee et al. 2007; Mathieson and Wilson 2010). Extensive studies of experimental schistosomiasis, mostly on murine *S. mansoni*, have revealed that granuloma formation is attributable to a vigorous CD4 + Th2-driven response, akin to a form of delayed-type hypersensitivity, that is tightly regulated by various cell populations, cytokines, and chemokines (Wynn et al. 2004).

Although *Schistosoma* periovular granulomas seem detrimental to the host, it is evident that they serve an indispensable host-protective function, especially during *S. mansoni* infection. Schistosome eggs and their secreted materials are a continuous antigenic stimulus for the immune system. If these antigens are not sequestered or neutralized effectively, they can harm the surrounding tissues, with hepatocytes



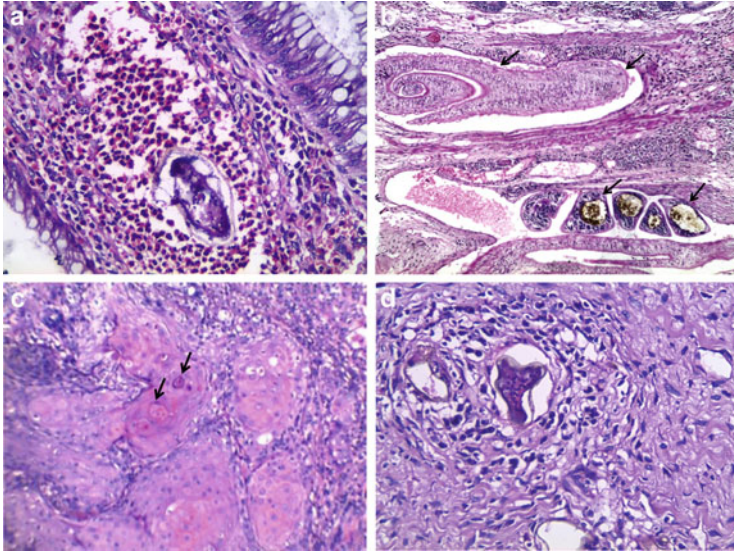
being particularly sensitive to toxins secreted by the eggs. Hence, granuloma formation seems to be a compromise, which allows the host to live with the infection for a long time. Hypothetically, the negative aspects associated with granulomas (fibrosis, portal hypertension) represent a better alternative, for host and parasite, than that of the host dying soon after parasite egg production (Pearce and MacDonald 2002; Wilson et al. 2007; Burke et al. 2009).

Histologically, five types of granuloma could be identified during the evolution of *Schistosoma*-induced granulomatous reaction irrespective of the anatomical site (Fig. 3.1), as indicated by early studies in mice, in rhesus monkeys (Hsu et al. 1972), and, later, in pigs (Hurst et al. 2000): the weakly reactive, exudative, exudative-productive, productive, and involucional stages (Hurst et al. 2000). Initially, there is accumulation of mononuclear cells, neutrophils, and eosinophils around the eggs (weakly reactive), which increases to form a microabscess in the exudative stage. Fibrinoid material is deposited around the eggs (Hoepli-Splendore reaction) (Lucas 2002). In the exudative-productive stage, histiocytes, epithelioid cells, and foreign body giant cells begin to replace the leucocytic zone. Fibroblasts form a rim around the granuloma. In the productive granuloma, the eggshells are disintegrated and the cellular elements are replaced by fibroblasts with deposition of collagen. Macrophages, lymphocytes, plasma cells, and few eosinophils are still found at the periphery. Finally, the involucional stage is characterized by marked shrinkage of granuloma which is replaced by hyalinized collagen fibres. Eggs may, by then, become calcified (Hsu et al. 1972; Hurst et al. 2000).

An effective T-cell response is known to be crucial for the development of the granulomatous response and host survival. Nude mice infected with a Chinese strain of *S. japonicum* supported normal parasite survival and fecundity, although transitory growth retardation was observed during the early stage of infection (Cheng et al. 2008). Moreover, these T-cell-deprived mice developed severe necrosis around the eggs in the liver, a situation similar to the T-cell-deprived mice infected with *S. mansoni*. Interestingly, B-cell function is required for the development of *S. japonicum* egg-induced granuloma in early infection (Ji et al. 2008). OBF-1 knockout mice and  $\mu$ MT mice, both with impaired B-cell development, developed significantly smaller hepatic granulomas at 5 weeks postinfection compared to their wild-type counterparts. In contrast, they displayed no significant difference in granuloma pathology at 8 weeks postinfection. This is in agreement with some studies on *S. mansoni*, also using B-cell-deficient mouse models, which have suggested that B cells are required for Th2 T-cell responses but not for granuloma formation late in infection (Ji et al. 2008).

Elegant studies with IL-13- and IL-4-deficient and IL-13/IL-4 doubly deficient mice have demonstrated that IL-4 launches the development of granulomatous inflammation, whereas IL-13 is the central profibrotic cytokine in the development of schistosome-induced liver fibrosis (Fallon et al 2000). Likewise, there are correlations between severity of hepatic fibrosis and levels of IL-13 expressed by peripheral blood mononuclear cells from individuals with chronic schistosomiasis mansoni (Oliveira et al. 2006). Typically, IL-4 determines the granuloma size, induces the proliferation of Th2 cytokine-producing lymphocytes, and is important





**Fig. 3.1** (a) Early “exudative” periovular schistosomal granuloma in colonic mucosa. Note the prominent eosinophilic infiltrate ( $\times 400$ ). (b) Sections of intravascular adult *Schistosoma* worms (arrows) in a resected colonic polyp from an Egyptian patient ( $\times 200$ ). (c) Squameous cell carcinoma of urinary bladder on top of schistosomiasis in an Egyptian patient. Note *Schistosoma* eggs (arrows) ( $\times 200$ ). (d) *Schistosoma* eggs in a resected ovarian adenofibroma from an Egyptian female, a common incidental finding in Egyptian patients ( $\times 400$ )

for the production of IL-5 and IL-13 by granuloma-associated cells (Cheever et al. 1994). Furthermore, IL-4 is not required for the development of fibrosis but enhances the fibrogenic effects of IL-13. As well as enhancing fibrosis, IL-13 has an additive effect with IL-4 in the development of the Th2-dominant, eosinophil-rich, granulomatous reaction (Fallon et al. 2000). Additionally, IL-5 is required for the recruitment of eosinophils to the granulomatous response as granulomas in mice deficient in IL-5 are virtually devoid of these cells (Cheever et al. 1991). Eosinophils are an important source of Th2 cytokines such as IL-13, and thus, IL-5 indirectly contributes to the polarization of the immune response through the recruitment of these cells (Rumbley et al. 1999; Burke et al. 2009). In contrast, the egg-induced Th2 immune responses and hepatic fibrosis are counter-regulated by IL-10, IL-12, and IFN- $\gamma$  (Hoffmann et al. 2002).

### 3.4.2.2 Immunomodulation of the Granulomatous Response

In chronic human infections, two paradoxical situations exist: on the one hand, the older indigenous individuals would harbour fewer worms probably due to the development of concomitant immunity and the fecundity of female worms

diminishes. Also, the newly formed granulomas become smaller in size. On the other hand, the granulomas will heal by fibrosis that could, over time, be dense enough to cause morbidity and irreversible sequelae (Butterworth and Thomas 1999).

Strict regulation of the Th1, Th2, and possibly Th17 cytokine responses generated during schistosome infection is essential to prevent excessive pathology. In experimental *S. mansoni* infection, CD4+ CD25+ Foxp3+ regulatory T-cells (Tregs) appear to regulate schistosome egg-induced immunopathology (Singh et al. 2005; Taylor et al. 2006). Thus, at 8 weeks after infection by *S. mansoni*, Foxp3 gene expression of splenocytes was similar to that of naive mice, but increased fourfold by 16 weeks. In contrast, granulomatous livers at 8 and 16 weeks showed 10- and 30-fold increase, respectively, in gene expression compared with the normal liver. The percentage of granuloma CD4+ CD25+ T cells rose from 12 % at 8 weeks to 88 % at 16 weeks of the infection. Moreover, retroviral transfer of the Foxp3 gene at the onset of granuloma formation enhanced fourfold Foxp3 expression in the granuloma CD4+ CD25+ T cells and strongly suppressed full granuloma development (Singh et al. 2005).

Several other mechanisms are thought to be involved in the down-modulatory process during *Schistosoma* infection. Well-designed studies in murine schistosomiasis have revealed that IL-13R $\alpha$ 2 is essential for the downregulation of the granulomatous response and is pivotal in the control of IL-13-mediated fibrosis. IL-13R $\alpha$ 2 acts as a potent decoy receptor, competing with IL-13R $\alpha$ 1 for binding of IL-13 and preventing signalling through the IL-4/IL-13R $\alpha$ 1 receptor complex (Wilson et al. 2007; Burke et al. 2009). A role for apoptosis, particularly apoptosis by neglect of CD4+ T cells, has been suggested to contribute to the down-modulation of the granulomatous response (Rutitzky et al. 2003). Furthermore, B-cell-mediated FcR-dependent signalling has also been implicated in the downregulation of the Th2 response as mice deficient in B lymphocytes or the Fc receptor exhibited marked exacerbation of granulomatous inflammation (Jankovic et al. 1998). Interestingly, some sort of immunomodulation, regarding the future immune and fibrogenic responses, occurs in offsprings born to experimentally *Schistosoma*-infected hosts in case of postnatal exposure to the parasite (Othman et al. 2010). Perinatal immunological sensitization may occur by transplacental and/or transmammary passage of schistosome antigens, anti-idiotypic antibodies, or immune complexes to the offsprings.

### 3.4.2.3 Other Immune-Mediated Phenomena in Man

Immune complex glomerulonephritis may occur in all types of *Schistosoma* infection but most commonly with *S. mansoni*, and the basic lesion is mesangioproliferative or membranoproliferative glomerulonephritis. Immunofluorescence and electron microscopy reveal the presence of immune complexes containing IgM, IgG, IgA, IgE, complement components, and schistosomal antigens in the mesangium and along the endothelial side of the capillary wall (van Velthuysen and Florquin 2000). Genetic and environmental factors, e.g. chronic salmonellosis,

are implicated in the pathogenesis of renal disease. The disease is manifested by proteinuria, hypertension, or nephrotic syndrome. It occurs years after the development of hepatosplenic disease, and it may be attributable, at least in part, to loss of immune complex clearing function of hepatic macrophages due to portosystemic shunting. Amyloid deposition in the kidney occurs infrequently in all types of schistosomiasis and may lead to nephrotic syndrome (van Velthuysen and Florquin 2000; Barsoum 2013). Moreover, *Schistosoma*-induced arthropathy has probably an underlying allergic immune mechanism (Saidenberg-Kermanac'h et al. 2005).

### **3.5 Pathogenesis and Clinical Features of Schistosomiasis**

#### ***3.5.1 Stage of Invasion (Cercarial Dermatitis)***

Penetration of human skin by cercariae of human schistosomes causes allergic reaction and dermatitis. There is erythema, accompanied by maculopapular and sometimes vesicular eruption. Scratching and secondary bacterial infection may lead to pustule formation. Exposure to cercariae of avian or bovine schistosomes, even for the first time, may lead to severe dermatitis (swimmer's itch). Treatment consists mainly of avoidance. Local and systemic antihistamines may be needed, as well as antibiotics (Butterworth and Thomas 1999).

Passage of schistosomula through the lungs and the liver may cause fever, cough, pneumonitis, and abdominal symptoms.

#### ***3.5.2 Stage of Maturation (Katayama Fever, Acute Schistosomiasis)***

This stage coincides with maturation of adult worms and start of deposition of eggs. The patient may experience fever, rigors, headache, dry cough, muscle and joint pain, hepatosplenomegaly, abdominal pain, lymphadenopathy, skin rash, eosinophilia, and patchy pulmonary infiltrates on chest radiograph. Severe central nervous system involvement may occur. The diagnosis relies on serological tests and finding eggs in the excreta (Butterworth and Thomas 1999; Gryseels et al. 2006; Ross et al. 2007). Treatment consists of schistosomicidal drugs with or without steroids. Artemether treatment given early after exposure may decrease the risk of Katayama fever (Ross et al. 2007; Jauréguiberry et al. 2010).

### **3.5.3 Stage of Established Infection**

The eggs of *Schistosoma* deposited in the tissues result in granuloma formation. In *S. haematobium* infection, deposition of eggs in the wall of the urinary bladder results in granulomatous inflammatory reaction and appearance of pseudotubercles. The patient suffers from cystitis and haematuria which is typically terminal but may be total. Haematuria is considered a normal developmental event in adolescent males in some regions of Africa. Symptoms of cystitis include suprapubic discomfort or pain, dysuria, and frequency (Bourée 2005). In *S. mansoni* and *S. japonicum*, deposition of eggs in the wall of the large intestine especially in the mucosa and submucosa results in granuloma formation and ulceration. The patient may suffer from chronic or intermittent abdominal pain and discomfort, loss of appetite, diarrhoea, and passage of blood and mucus in stool (schistosomal dysentery). This stage, unfortunately, may be asymptomatic or oligo-symptomatic, thereby the disease may progress unchecked until complications supervene (Butterworth and Thomas 1999; Gryseels et al. 2006).

### **3.5.4 Stage of Late Infection and Sequelae**

The complications are mainly due to healing of schistosomal lesions by fibrosis. The damage produced at this stage is irreversible due to organ damage by fibrosis and vascular changes.

#### **3.5.4.1 Genitourinary Schistosomiasis**

##### **Obstructive Uropathy**

Healing of granulomatous lesions in the wall of the urinary bladder and lower ends of the ureters results in fibrosis and urinary obstruction. Various characteristic pathological lesions may appear such as ulceration, sandy patches, cystitis cystica, and calcification. The bladder may become rigid and contracted with reduced capacity. Moreover, urinary stasis predisposes to urinary tract infection and calculus formation which further aggravate the urinary obstruction. The end result is hydroureter and hydronephrosis. The progressive obstructive uropathy could ultimately lead to end-stage renal failure (Butterworth and Thomas 1999; Bourée 2005; Barsoum 2013).

## Bladder Cancer

The mucosa of the urinary bladder undergoes squamous metaplasia due to chronic inflammation. This predisposes to squamous cell carcinoma of the bladder. The typical histopathological lesion reported in many studies over the years is a squamous cell carcinoma in roughly 60 % of cases. Other histological types include transitional cell carcinoma (20 %), adenocarcinoma (10 %), and mixed (10 %). Schistosomal ova were detected in more than 85 % of bladder cancers in an Egyptian series of 1026 cases subjected to surgical cystectomy (Ghoneim et al 1997). The tumour, particularly when of the squamous cell type, remains localized for a long time before spreading to the surrounding pelvic tissues or a distant site, owing to the occlusion of lymphatics by the fibrotic process (Barsoum 2013).

Associated bacterial and viral infections, rather than parasitic products, are suggested to be the main pathogenic factors. Associated infection with human papillomavirus has received considerable recent attention in this respect (el-Mawla et al. 2001), being encountered in about one-fourth of cases. Moreover, specific p53 gene mutations have been observed in one-third of cases (Warren et al. 1995), being attributed to the effect of neutrophil-generated reactive oxygen molecules, the cleavage of conjugated urinary carcinogens, or the production of nitrosamines by bacterial enzymes (Mostafa et al. 1999).

## Other Lesions

Eggs may be deposited in the seminal vesicles, prostate, and spermatic cord in males leading to funiculitis, epididymitis, prostatitis, haemospermia, and rarely infertility. In females the eggs may reach the vagina, uterine cervix, and fallopian tubes, resulting in inflammation and fibrosis that may predispose to infertility or ectopic pregnancy. Schistosomal lesions of the genital organs may increase chances of transmission of sexually transmitted pathogens including HIV. Moreover, lesions in the uterine cervix may be mistaken for carcinoma (Bourée 2005; Barsoum 2013).

### 3.5.4.2 Intestinal Schistosomiasis

Schistosomiasis *mansoni* causes patchy fibrosis of the wall of the intestine. Strictures, sinuses, and fistulae may occur. It may lead also to colonic polyposis resulting in bleeding, anaemia, protein loss, and hypoproteinaemia. Polyps range in size from 2 to 20 mm and may be sessile, pedunculated, or showing a cauliflower appearance. They are mainly concentrated in the distal colon, and they range from few to very numerous polyps. The overlying mucosa is usually redder than the surrounding mucosa due to severe congestion and due to focal haemorrhages. Ulceration is common in rectal polyps; the ulcerated areas appear dusky to blackish grey in

colour caused by superficial haemorrhage and are frequently secondarily infected (El-Garem 1998; Elbaz and Esmat 2013).

*S. japonicum* affects similarly the large intestine as well as the stomach. *S. mekongi* gives manifestations that are similar to those of *S. japonicum* but usually milder. *S. intercalatum* infection is usually asymptomatic or causes mild intestinal symptoms. The latter may also infrequently give rise to urinary manifestations (Davis 2009).

There are contradictory reports on the contribution of *S. japonicum* to the aetiopathogenesis of colorectal or primary liver cancer (Davis 2009). The situation is more ambiguous for *S. mansoni*. The anecdotal reports of the association of schistosomiasis mansoni and colorectal carcinoma do not differentiate between direct causal relationship and mere accompaniment (Salim et al. 2010). To date, the carcinogenicity of both species is not proved, and the issue awaits further well-designed research for clarification.

### 3.5.4.3 Hepatic Schistosomiasis

In case of *S. mansoni* and *S. japonicum*, more than 50 % of eggs are not passed in the faeces and are retained in the tissues. Eggs swept to the liver via the portal circulation induce granuloma formation and periportal hepatic fibrosis, ending, in some cases, in presinusoidal portal hypertension (Fig. 3.2). Portal hypertension leads to the opening of portosystemic anastomotic venous channels resulting in oesophageal (and gastric) varices, secondary haemorrhoids, and ascites. Bleeding from oesophageal varices may lead to fatal haematemesis and/or melena. Splenomegaly occurs due to passive congestion secondary to portal hypertension and reticuloendothelial and lymphoid hyperplasia. The spleen is markedly enlarged, firm, smooth, and non-tender. A huge spleen may cause discomfort and dyspepsia or pain in the case of perisplenitis or infarction. Hypersplenism may also develop (Butterworth and Thomas 1999; Davis 2009; Elbaz and Esmat 2013).

Radiographic studies indicate amputation of the large portal veins, development of collateral veins, arteriportal venous shunts, and diminished hepatic arterial diameters (Da Silva and Carrilho 1992). When portal fibrosis is established, with its associated distortion of vascular architecture, the incoming eggs can pass through collateral veins around the large portal veins and via the granuloma/fibrosis sequence; this results in progressively expanding tracts of collagen that characterize the clay-pipestem pattern of fibrosis, originally described by Symmers (Lucas 2002). *S. japonicum*, owing to smaller size of its eggs, produces periportal fibrosis that affects the peripheral and central zones of the liver, whereas *S. mansoni* affects only the central zones (Burke et al. 2009).

Hepatic function is well preserved in pure schistosomal fibrosis until very late. Decompensated hepatosplenic disease with stigmata of liver cell failure, especially ascites, may supervene. This may occur due to malnutrition, severe collagen deposition in the space of Disse, ischaemic damage from repeated variceal bleeding, and severe distortion of arteriovenous relationships in the portal tracts. Alcohol

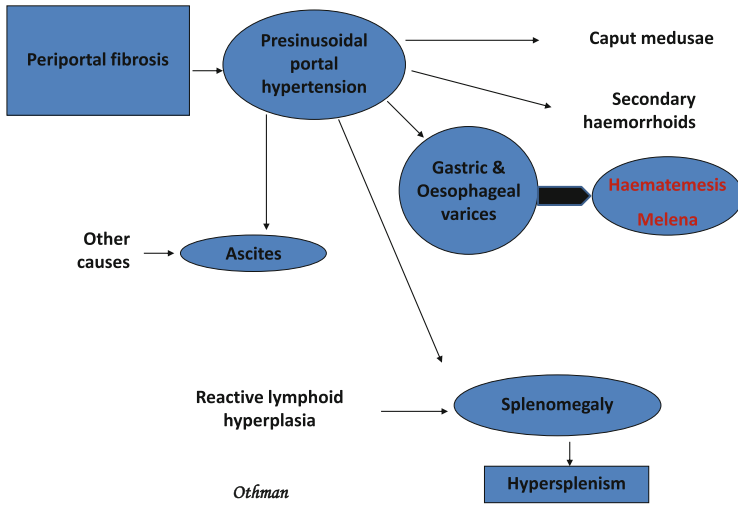


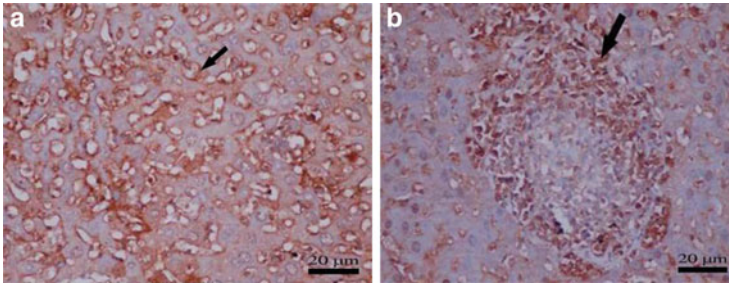
Fig. 3.2 Pathogenesis of hepatic schistosomiasis

abuse and viral hepatitis types B, C, D, or E are additional complicating factors (Watt et al. 1991; Davis 2009).

Fibrosis is the ultimate sequel and a major culprit of chronic hepatic schistosomiasis. Apparently, hepatic stellate cells (HSCs) play a major role in the process of fibrous tissue formation in the liver. They are responsible for the synthesis of components of extracellular matrix and several types of collagen as well as fibrogenic cytokines, matrix metalloproteinases, and tissue inhibitors of metalloproteinases that contribute to the remodelling of fibrous tissue (Parola and Robino 2001). HSCs reside in the Disse’s spaces of the liver sinusoids (Fig. 3.3), and they constitute a minor cell type, roughly 5–8 % of the total liver cells (Maubach et al. 2006). Following chronic injury, HSCs differentiate into myofibroblast-like cells, acquiring contractile and fibrogenic properties (Zhang et al. 2006). Remarkably, HSCs affect adversely the hepatic microcirculation. When activated, they transform into myofibroblasts that contract around the hepatic sinusoids, increasing the vascular resistance and contributing to portal hypertension (Friedman 2000). The different stimuli that initiate and perpetuate HSC activation in chronic liver disease are poorly understood, but the role of oxidative stress seems crucial. This explains the multitude of trials of administration of exogenous antioxidants (e.g. coenzyme Q10, melatonin, vitamin E, silymarin, and molecular hydrogen) in an attempt to reverse or limit *Schistosoma*-induced liver fibrosis (Gharib et al. 2001; El-Sokkary et al. 2002; Othman et al. 2008).

Several immunological and non-immunological determinants govern the progression of schistosomal fibrosis. The role of cytokines, such as IL-4 and IL-13, has been discussed before. Regulatory cytokines such as IL-10, IL-12, and transforming growth factor  $\beta$  also influence the fibrogenic response of the host (Othman et al. 2010). Alternatively activated macrophages (aaM $\Phi$ ) are hypothesized to





**Fig. 3.3** Hepatic immunohistochemical stain for HSCs in *Schistosoma*-infected mouse showing (a) sinusoidal “arrow” and (b) mesenchymal “arrow” HSCs (immunoperoxidase GFAP  $\times 400$ )

contribute to schistosome-induced fibrosis in murine schistosomiasis (Wynn et al. 2004; Wilson et al. 2007; Stavitsky 2004). Alternative activation of macrophages is induced by Th2 responses and promotes collagen synthesis and fibrogenesis via the metabolism of L-arginine to proline and polyamine by arginase-1. Finally, the genetic constitution of the host was found to determine the propensity to fibrosis. In Egypt, patients with human leucocyte antigen (HLA)-A1 and HLA-B5 have been associated with severe hepatosplenic schistosomiasis mansoni, while in the case of *S. japonicum* infection, HLA-DR and HLA-DQ have been implicated in the differential regulation of immune responses to egg antigens (Butterworth and Thomas 1999).

Research has indicated that over time schistosomal fibrosis becomes established owing to cross-linking of collagen fibres, rather than the difference in collagen isotypes deposited early or late in infection (Ricard-Blum et al. 1992). When the fibrosis is recent in early infection, a process of collagen breakdown occurs with therapy. This breakdown is almost complete, as well as rapid and abrupt. On the other hand, long-standing fibrosis in chronic infection could also undergo regression with therapy, but the process is slow and gradual. Regression of fibrosis entails collagen degradation and vascular remodelling which includes neovascularization (Andrade 2008). The process of regression of schistosomal fibrosis has been demonstrated both in humans and experimental animals. In humans, it can be translated by regression of splenomegaly and oesophageal varices.

Ultrasonography has greatly facilitated the assessment of schistosomal hepatic fibrosis, splenomegaly, portal vein dimensions and the presence of collateral vessels (Lambertucci et al. 2008). It helps to assess the degree of periportal fibrosis by measuring the portal tract thickness: Grade I if thickness is 3–5 mm, Grade II if it is 5–7 mm, and Grade III if it is more than 7 mm. This method reflects the haemodynamic changes and provides a good estimate of the clinical status of patients who have periportal fibrosis (Abdel-Wahab et al. 1992). Notably, in an attempt to harmonize the practices in clinical trials and evaluation of therapeutic measures, WHO elaborated a standard ultrasound scoring protocol (Niamey–Belo Horizonte) which includes a qualitative assessment of liver parenchyma (according to reference patterns A to F) (King et al. 2003). Portal hypertension is suspected

when dilatation of one or more of the portal, mesenteric, and splenic veins is detected. For the collateral vessels, the most commonly described are the left and right gastric, the short gastric, the paraumbilical, the splenointercostal, and the splenorenal veins (Lambertucci et al. 2008; Pinto-Silva et al. 2010). Lastly, the hepatic veins in schistosomiasis can be assessed ultrasonographically. They remain patent with normal phasic flow as the disease evolves, which is different from liver cirrhosis (Elbaz and Esmat 2013).

### Interactions with Viral Hepatitis

Co-infection with either hepatitis B virus (HBV) or hepatitis C virus (HCV) is very common since the regions with a high prevalence of schistosomiasis usually have a high endemicity of chronic viral hepatitis as well (Elbaz and Esmat 2013). An important cause of the extraordinary high prevalence of HCV in Egypt was the establishment of a large reservoir of infection as a result of the mass parenteral chemotherapy campaigns against schistosomiasis that ended in the 1980s (Frank et al. 2000). The association between both schistosomiasis and HCV is known to cause earlier deterioration of hepatic functions and more severe morbidity. The liver is the principal site for both HCV replication and egg deposition. The latter downregulates the local immune responses in the liver (Lundy et al. 2001) and results in the suppression of the intrahepatic bystander immune response to HCV. Moreover, this co-infection can produce a unique clinical, virological, and histological pattern manifested by viral persistence with high HCV RNA titres, higher necro-inflammatory and fibrosis scores in liver biopsy specimens as well as poor response to interferon therapy, and rapid progression of liver fibrosis (Kamal et al. 2006).

#### 3.5.4.4 Anaemia

Schistosomiasis-associated anaemia is well documented and the cause is multifactorial. Elevated levels of proinflammatory cytokines, such as TNF- $\alpha$  and IL-6, resulting in anaemia of inflammation, are thought to play a central role. Moreover, for intestinal schistosome species, microcytic hypochromic anaemia may occur due to blood loss in the stool and consumption of blood by adult worms. Colonic polyposis also results in bleeding, protein loss, and anaemia. Hypersplenism, bleeding oesophageal varices, malnutrition, and autoimmune haemolysis are additional factors. In schistosomiasis haematobium, anaemia may develop due to blood loss in urine (Friedman et al. 2005; Davis 2009). Interestingly, *S. mansoni* SEA has recently been found to trigger erythrocyte cell death in an animal model, thus contributing to the development of anaemia (Kasinathan and Greenberg 2010). Anaemia in turn has been associated with wasting in adults and growth retardation and cognitive impairment in children.

### 3.5.4.5 Neuroschistosomiasis

Although uncommon in comparison to the total toll of schistosomal disease, neuroschistosomiasis is not uncommon and is probably underrecognized. Spinal cord schistosomiasis, especially due to *S. mansoni*, is considered as a primary cause of spinal cord parasitic infection in Egypt (Badr et al. 2011).

Central nervous system (CNS) disease may occur in all *Schistosoma* spp. during the initial stage of maturation (acute schistosomiasis) particularly in case of *S. japonicum*. The disease tends to be more common and severe in non-endemic exposed individuals. It is probably due to immunologically mediated vasculitis. In the chronic stage, the pathology is due to passage of eggs of *Schistosoma* to CNS where they induce space-occupying granulomatous reactions (Ferrari et al. 2008; Ferrari and Moreira 2011).

*Schistosoma* eggs (or occasionally adults) may reach the CNS via two routes: the first is through Batson's vertebral venous plexus which connects the portal venous system and inferior venae cavae to the spinal cord and cerebral veins. This route permits both anomalous migrations of adult worms in copula to sites close to the CNS followed by in situ deposition and, occasionally, massive embolization of eggs from the portal mesenteric and pelvic venous system towards the CNS. High intra-abdominal pressure, e.g. during defecation and coughing, increases the chance of retrograde flow. The second route is via the arterial system either directly as in *S. haematobium* or after development of portocaval anastomoses secondary to portal hypertension in *S. mansoni* (Katchanov and Nawa 2010; Ferrari and Moreira 2011).

In the chronic stage of infection, cerebral disease is more common in *S. japonicum* than in other species and most commonly presents with seizures. On the other hand, *S. mansoni* and *S. haematobium* (to a lesser extent) affect more commonly the spinal cord leading to paresis, radicular symptoms, sphincteric problems, and cauda equina syndrome (Hughes and Biggs 2002). Schistosomal myelopathy has been reported to occur 38 days to 6 years after infection and is acute or subacute with rapidly progressive neurological deficit over the first 24 h. In one review of 26 patients with spinal cord disease, 11.5 % died, 34.6 % remained paraplegic, and 54 % showed moderate to good improvement with therapy (Scrimgeour and Gajdusek 1985).

The diagnosis relies on clinical presentation, demonstration of schistosomal infection by microscopy and serological methods, imaging features, and finally exclusion of other causes of myelopathy (Lambertucci et al. 2008). Cerebrospinal fluid (CSF) examination may be normal but may show increased protein and pleocytosis in the majority, but eosinophilia in only one-quarter of cases. Eggs were never found in CSF (Hughes and Biggs 2002). Histopathology of specimens obtained after surgical removal or biopsy provides definitive diagnosis.

In cerebral disease, CT and MRI usually show a nonspecific tumour-like lesion surrounded by oedema, associated with mass effect and heterogeneous contrast enhancement. The borders are often irregular and poorly defined. Further, MRI is

very sensitive in the detection of abnormalities in patients with spinal disease, but the alterations are nonspecific. The most common findings are signal hyperintensity on T2-weighted images, enlargement of the spinal cord (particularly lower cord and conus medullaris), thickening of the spinal roots (especially cauda equina roots), and a heterogeneous pattern of contrast enhancement on T1-weighted images (Silva et al. 2004; Lambertucci et al. 2008).

#### 3.5.4.6 Cardiopulmonary Schistosomiasis

Embolization of eggs into the capillary bed of the lungs may occur via systemic circulation in *S. haematobium* infection and in intestinal species especially *S. mansoni* after development of portosystemic venous anastomoses secondary to portal hypertension. In an early study in Upper Egypt, pulmonary schistosomiasis was associated with *S. haematobium* infection in 58 % of cases, *S. mansoni* infection in 31 % of cases, and mixed infection in 11 % of cases (Shaw and Ghareeb 1938). Furthermore, the prevalence of pulmonary hypertension in the patients with schistosomal liver fibrosis was found to be 10.7 % in one Brazilian study (Ferreira et al. 2009).

The eggs induce pulmonary granuloma formation and fibrosis, and vascular changes occur in the adjacent vessels, resulting in necrotizing arteritis, thrombi in the affected vessels, medial hypertrophy, and intimal proliferation. Plexiform lesions develop in severe cases where dilated arterioles and venules are detected. The loss of vascular structures can lead eventually to pulmonary hypertension with or without cor pulmonale (Gutierrez 2000). Clinically, the patient may complain of fatigue, exertional dyspnoea, chest pain, cough with occasional haemoptysis, oedema, and later central cyanosis and clubbing of fingers. Electrocardiographic and radiological abnormalities are observed.

#### 3.5.4.7 *Schistosoma*–*Salmonella* Interactions

Concurrent *Schistosoma*–*Salmonella* infections occur when enteroinvasive *Salmonella* enter the systemic circulation and attach to the tegument of adult *Schistosoma* present in the mesenteric vasculature (LoVerde et al. 1980; Melhem and LoVerde 1984). This interaction apparently provides a refuge in which the bacterium can evade systemic antibiotic therapy. Chronic bacteraemic salmonellosis is an individualized clinical entity characterized by prolonged fever with enlargement of the liver and spleen that occurs in *Schistosoma*-infected individuals who are co-infected with *Salmonella*. Furthermore, therapy with the anthelmintic praziquantel can lead to a massive release of schistosome-associated *Salmonella* causing bacteraemia if the appropriate antibiotic is not co-administered to co-infected individuals. Finally, the use of ineffective antibiotics contributes to antibiotic resistance development and the phenomenon of bacterial persistence (Barnhill et al. 2011).

### 3.5.4.8 Other Manifestations

Deposition of eggs in the skin causes papular dermatitis. Growth retardation and infantilism may occur in schistosomiasis partly due to decreased levels of somatomedins. Moreover, polyarthritis may occur in *S. mansoni* infection, while *S. haematobium* may cause osteomalacia resulting from tubular lesions in association with obstructive uropathy (Butterworth and Thomas 1999).

## 3.6 Diagnosis

### 3.6.1 Parasitological Techniques

The detection of eggs in stool or urine remains the “gold standard” for schistosomiasis diagnosis despite its lack of sensitivity because of its unsurpassed level of specificity. The shape, size, and spine of the eggs of the three major schistosome species are useful diagnostic features. The eggs of *S. japonicum* are round with a reduced lateral spine and are smaller in size ( $60 \times 100 \mu\text{m}$ ) than those of *S. mansoni* ( $61 \times 140 \mu\text{m}$ ; prominent lateral spine) and *S. haematobium* ( $62 \times 150 \mu\text{m}$ , prominent terminal spine), which are both ovoid. The 10 ml urine sedimentation and filtration techniques are reliable field diagnostic methods for *S. haematobium* (Dazo and Biles 1974). The Kato–Katz technique (Endriss et al. 2005) remains the method of choice for the diagnosis of intestinal schistosomiasis, despite its limited sensitivity in areas of low endemicity and the conspicuous intra- and inter-specimen variation of egg distribution and aggregation in faeces (Krauth et al. 2012). However, Berhe et al. (2004) reported that “examination of five Kato-Katz thick smears from one stool specimen using 41.7 mg template or three Kato-Katz thick smears from one stool specimen, and if these are negative, followed by examination of additional triplet Kato-Katz thick smears from subsequent day stool specimen can adequately assess individuals for infection status with *S. mansoni*”. Similar findings were recorded for *S. japonicum* parasitological diagnosis (Lin et al. 2008; Utzinger et al. 2011). The formol–ether concentration technique (Allen and Ridley 1970) is slightly more sensitive than the triplet Kato–Katz slides (Ebrahim et al. 1997) but less sensitive than the FLOTAC method (Cringoli 2006) which is recommended for highly accurate coprological diagnosis and for ascertaining cure following chemotherapy (Glinz et al. 2010; Utzinger et al. 2011). The Percoll separation technique (Eberl et al. 2002) could be used in hospitals and laboratories dedicated to research and clinical investigations.

In light infection, examination of rectal mucosa snips taken with a curette through a proctoscope is more sensitive than stool examination. Material thus obtained is examined as a crush preparation. Viability of eggs is confirmed by movement of the cilia of flame cells or of the miracidia within the eggshell (Davis 2009).

### 3.6.2 Immunological Techniques

Schistosomes elicit immune responses immediately upon host skin invasion and during development, maturation, copulation, and egg laying in blood vessels. Accordingly, antibody responses to different schistosome adult worm (SAWA)- or egg (SEA)-derived antigens or selected antigens, e.g. species-specific microsomal antigens, cathepsin B, etc., have been used for immunodiagnosis by indirect haemagglutination assay (IHA), enzyme-linked immunosorbent assay (ELISA), and/or dipstick dye immunoassay, all of which showed high sensitivity (Al-Sherbiny et al. 1999; Noya et al. 2002; Xiang et al. 2003; Zhou et al. 2007, 2008, 2011a). IHA is among a few commercial serodiagnostic kits for schistosomiasis and was shown to be ideal for assessing IgM and IgG responses in travellers to, but not residents of, endemic regions (van Gool et al. 2002; Yu et al. 2007; Zhou et al. 2007, 2008; Zhao et al. 2012a, b). Antibody detection methods are limited in their specificity as they do not allow discrimination between current and previous infection or reinfection and are thus not useful for assessing follow-up after chemotherapy (Zhou et al. 2011b).

Immunodiagnostic methods based on antigen detection have overcome some of these limitations. Worm antigens and egg-derived molecules are readily detectable in serum or plasma, faeces, urine, and even saliva, provided the availability of specific capture and detecting antibodies (Lei et al. 2011; Demerdash et al. 2013). Such immunodiagnostic methods are of importance not only for assessment of cure after treatment but even more importantly as the most reliable way for diagnosis of chronically infected patients who fail to excrete eggs in stool or urine (Enk et al. 2008; Zhou et al. 2011b). Research has primarily focused on two glycoproteins, circulating anodic antigen (CAA) and circulating cathodic antigen (CCA), which are released from the gut of viable developing larvae and adult worms and may thus provide information on active infection and intensity (van Dam et al. 1996). The assays require access to CAA- and CCA-specific antibodies (Al-Sherbiny et al. 1999); yet, commercial kits are becoming available and showed exquisitely high sensitivity and specificity for diagnosis of *S. mansoni* but not *S. haematobium* (Stothard et al. 2006; Ashton et al. 2011; Shane et al. 2011; Utzinger et al. 2011; Navaratnam et al. 2012; Tchuem Tchuente et al. 2012; Bergquist 2013; Coulibaly et al. 2013; Erko et al. 2013; Grenfell et al. 2013).

### 3.6.3 Molecular Techniques

Conventional or real-time polymerase chain reaction (PCR) amplification of repetitive elements specific to each schistosome species has been used for the diagnosis of *S. mansoni* (Pontes et al. 2002, 2003; Rabello et al. 2002; Abath et al. 2006; Gomes et al. 2006; Allam et al. 2009), *S. haematobium* (Abbasi et al. 2007; Obeng et al. 2008;

Aryeetey et al. 2013), and *S. japonicum* (Xu et al. 2010; Zhao et al. 2012a) using stool, urine, or serum samples.

## 3.7 Treatment of Schistosomiasis

### 3.7.1 Schistosomicidal Agents

#### 3.7.1.1 Praziquantel (PZQ)

Praziquantel is a pyrazino-isoquinoline derivative that is practically insoluble in water, sparingly soluble in ethanol, but very soluble in chloroform and dimethyl-sulfoxide. PZQ is the mainstay of antischistosomal therapy; it is effective against all five species of human schistosomes in a single oral dose of 40–60 mg/kg body weight, leading to cure rates of 60–90 % in different epidemiological settings (Davis 1993; Kumar and Gryseels 1994). Despite that the initial effects of the drug included a rapid influx of calcium into the worm and calcium-dependent muscle contraction and paralysis, the exact mode of action of PZQ is not known as yet (Greenberg 2005), and the PZQ receptor on schistosomes remains elusive. However, in vivo, PZQ-induced muscle contraction and tegumental lesions produce loss of attachment to the endothelial lining of veins and dislodgment to the liver. Host cells of the defence system attach to the tegumental vacuoles and start to penetrate the interior of the parasite early after treatment (Mehlhorn et al. 1981; Andrews 1985; Day et al. 1992). It has been recently shown that PZQ binds and polymerizes adult schistosome actin, which may account for some of its effects (Tallima and El Ridi 2007). Adverse effects include direct and dose-related effects such as nausea and abdominal pain, headache, and dizziness, as well as indirect effects attributable to the death of worms such as fever, urticaria, pruritus, rashes, arthralgia, myalgia, and eosinophilia (Tracy and Webster 2001). PZQ can be administered to pregnant women at any stage of pregnancy and during lactation, as benefit of treatment outweighs risk. Also, the drug can safely be administered to school-age children (WHO 2006).

Two problems are noteworthy with PZQ: first, the emergence of drug resistance—isolates with decreased sensitivity to praziquantel have been reported from different epidemiological settings, e.g. Egypt, Kenya, and Senegal (Ismail et al. 1996; Cioli et al. 2004; Melman et al. 2009), and may be harbingers of more to come. Second, the drug is not effective against juvenile forms of the parasite. It has little activity against eggs or immature worms (schistosomula) and cannot abort early infection. Therefore, patients treated early in their infection must be retreated with PZQ after the adult worms have matured (usually in 6–12 weeks) (Kappagoda et al. 2011).



### 3.7.1.2 Other Drugs

#### Oxamniquine

When administered orally, it is effective against *S. mansoni*, male worms being more affected than females, while it has no effect on *S. haematobium*. A single, two, or three oral doses of 20 mg/kg each are needed for a cure rate of 80–90 %, depending on the geographical region. It is now believed that oxamniquine undergoes esterification by a sulfotransferase uniquely present in sensitive schistosomes. The ester spontaneously dissociates, yielding an electrophilic reactant capable of alkylating schistosome DNA, with subsequent inhibition of DNA and RNA synthesis. The absence of this enzyme in mammals, including humans, explains the low toxicity of oxamniquine (Pica-Mattocchia and Cioli 1985). Oxamniquine is no longer used on a large scale (El Ridi and Tallima 2012).

#### Antimalarials

The antimalarial drug artemether, a methoxy derivative of artemisinin, has been shown to be active against *S. japonicum*, *S. mansoni*, and *S. haematobium* in experimentally infected animals (Xiao et al. 2002). Mefloquine, another antimalarial drug, was also found to have significant anti-schistosome activity in vitro, and in vivo as well, since a single dose (200 or 400 mg/kg), administered orally to mice infected with adult *S. mansoni*, resulted in worm burden reductions of 72.3–100 % (Keiser et al. 2009). It has been shown that artemether interacts with haemin to exert a toxic effect on schistosomes, while mefloquine is believed to inhibit haemozoin formation. Artemisinin derivatives are particularly effective against the immature stages of *S. japonicum*, *S. mansoni*, and possibly *S. haematobium*. Yet there are objections for use of antimalarials in the treatment of schistosomiasis for fear of development of artemisinin-resistant malaria (Gryseels et al. 2006; Utzinger et al. 2007).

The most successful plant product in the treatment of human schistosomiasis was myrrh, an oleo–gum–resin extracted from the stem of *Commiphora molmol* (Tonkal and Morsy 2008). The product is believed to affect schistosome musculature, leading to uncoupling of male and female couples and their extravasation to the liver (Badria et al. 2001). The product has been licensed for human use by the Egyptian Ministry of Health and introduced in the Egyptian market. However, conflicting reports on its efficacy shed doubts upon the usefulness of its use as a novel therapy for schistosomiasis (Osman et al. 2010).

### 3.7.1.3 Novel Therapeutic Approaches

A different approach to therapy of schistosomiasis has relied on plants known for medicinal effects. Extracts and oils of several medicinal plants have been tested for potential therapeutic activity against schistosome infection and have been exhaustively compiled in excellent reviews (e.g. Tagboto and Townson 2001; Yousif et al. 2007). Curcumin, the major constituent in the rhizome of *Curcuma longa*, has been shown to display potent schistosomicidal activities in vivo and in vitro against adult *S. mansoni* worms (El-Ansary et al. 2007; El-Banhawey et al. 2007).

An essential fatty acid, a component of our diet and cells, the polyunsaturated fatty acid arachidonic acid has been proposed as a remedy for schistosomiasis, due to its ability to activate the parasite tegument-bound neutral sphingomyelinase, with subsequent hydrolysis of the apical lipid bilayer sphingomyelin molecules, allowing access of specific antibody molecules, and eventual worm attrition. This concept was convincingly supported using larval and adult *S. mansoni* and *S. haematobium* worms in in vitro experiments and in vivo studies in inbred mice and outbred hamsters (El Ridi and Tallima 2013a, b).

Of great interest is the class of compounds targeting schistosome histone-modifying enzymes, namely, histone acetyltransferases and histone deacetylases, leading to parasite apoptosis and death in in vitro cultures (Pierce et al. 2011). Further, trioxaquines, which are currently in development for malaria, are hybrid molecules consisting of two pharmacophores, a trioxane and a 4-aminoquinoline moiety (Utzinger et al. 2011). These drugs, used at concentrations of 5–50 µg/ml, rapidly kill 21-day-old juvenile and 49-day-old adult *S. mansoni* in vitro. Therefore, these evidence-based findings confirm that peroxidic compounds are active against schistosomes and perhaps other trematodes (Keiser and Utzinger 2007; Xiao et al. 2007).

Recently, there has been an interest in multidrug transporters, members of ATP-binding cassette (ABC) superfamily of efflux transporters, such as P-glycoprotein (Pgp, ABCB1), which have been associated with drug resistance in parasites, including helminths such as schistosomes (Greenberg 2013). The role of schistosome ABC transporters in regulating drug susceptibility and in normal schistosome physiology, including reproduction and excretory activity, has been recently explored. For example, *S. mansoni* expresses higher levels of multidrug resistance-associated protein 1 (SmMRP1) in juvenile worms and in response to praziquantel (Kasinathan et al. 2010). Greenberg (2013) postulated that schistosome ABC transporters could be useful targets for compounds that enhance the effectiveness of current therapeutics as well as for agents that act as antischistosomes on their own.

## Follow-Up Therapy

Parasitological cure has to be confirmed 6–8 weeks and 4–6 months after therapy. Finding viable eggs at 4–6 months indicates either reinfection, therapeutic failure, or that treatment was given too early after infection. Other parameters of cure include improvement of symptomatology and improvement of radiological, ultrasonographic, and endoscopic findings (Davis 2009). Antibody titres, and eosinophilia if present, decline with therapy. However, there may be transient eosinophilia and rise in antibody titres in the first 2 weeks after therapy (Bourée 2005). Antigen detection, if available, is an excellent test for establishing cure.

### ***3.7.2 Symptomatic Therapy and Management of Complications***

For the prevention of bleeding from oesophageal varices due to schistosomal portal hypertension, pharmacotherapy in the form of beta blockers is used together with endoscopic treatment in the form of sclerotherapy or much better band ligation. For the management of acute variceal bleeding, resuscitation is mandatory together with the administration of vasoactive drugs (e.g. terlipressin, somatostatin, or octreotide). Endoscopic treatment particularly band ligation is effective in controlling bleeding. Other procedures for portal hypertension include the minimally invasive transjugular intrahepatic portosystemic shunt (TIPS). Surgery is reserved for those patients who fail to respond to endoscopic therapy or in case of symptomatic hypersplenism and includes oesophagogastric devascularization or shunt procedure together with splenectomy (Bosch et al. 2008; Rajekar et al. 2011). Colonoscopic polypectomy is safe and effective and may be required along with medical therapy to achieve complete symptom relief and prevent complications (Mostafa et al. 1990; Elbaz and Esmat 2013).

Obstructive uropathy is usually managed conservatively. However, a damaged kidney may necessitate nephrectomy or nephrostomy. Corrective surgery may be needed for contracted bladder and ureteric obstruction. Carcinoma of the bladder is treated through the conventional procedures of tumour management. Total or partial cystectomy is usually needed. Schistosomal bladder cancer responds less favourably to radiotherapy (Bourée 2005).

## **3.8 Prognosis**

Schistosomiasis remains a major cause of morbidity in tropical and subtropical countries (Engels et al. 2002). Clinical observations in schistosomiasis endemic areas show that major complications of *Schistosoma* infection develop in

approximately 4–12 % of the population, while the majority of infected people remain asymptomatic or exhibit mild nonspecific symptoms (Tachon and Borojevic 1978). Intensity and duration of the infection are major determinants, but other factors are also involved. These include genetic background of the host, nutritional status, parasite strain differences, and frequency of infection. Maternal infection status—in that offspring of the infected mothers may be primed to mount a modulated type of response at first infection—has been proposed by many authors in order to explain the individual differences (Butterworth and Thomas 1999).

Additionally, schistosomiasis does not cause the annual loss of between 1.7 and 4.5 (Steinmann et al. 2006) but between 24 and 56 (King 2010) and up to 70 (Gray et al. 2010; Siddiqui et al. 2011) million disability-adjusted life years. Unlike malaria, tuberculosis, and HIV/AIDS, schistosomiasis and a host of other helminthic, bacterial, protozoan, and viral diseases remain truly neglected (Utzinger et al. 2011). The close link with poverty and lack of resources, geographical isolation, underestimated global burden, stigmatization, lack of political voice of those affected, and the absence of an established global funding mechanism are some of the factors that explain the general neglect of schistosomiasis (Molyneux 2008; Gray et al. 2010; Utzinger et al. 2011).

At the individual level, antischistosomal therapy given early could lead to resolution of schistosomal lesions and could halt the progression of the disease provided that reexposure to infection is avoided. In case of established fibrosis, antischistosomal therapy is given to prevent further damage, but the current morbidity has to be addressed appropriately (Butterworth and Thomas 1999). However, there is evidence of regression of hepatosplenic disease with therapy (Ohmae et al. 1992; Butterworth and Thomas 1999; Lucas 2002; Andrade 2008).

## 3.9 Prevention and Control

### 3.9.1 Preventive Chemotherapy

WHO (2006) recommended PZQ as the basis of preventive chemotherapy in schistosomiasis. PZQ-based preventive therapy is a cost-effective public health tool that aims at morbidity control: periodic treatment of at-risk populations will cure subtle morbidity and prevent infected individuals from developing severe, late-stage morbidity due to schistosomiasis. For the assessment of prevalence of *Schistosoma* infection in a suspected endemic area, schoolchildren are examined. A few schools are selected close to the water and some a little further away, and 50 students of the upper classes in each school are examined either by stool examination (Kato–Katz method) for intestinal schistosomiasis or by search for haematuria (via a questionnaire or urine examination) or urine analysis for ova for urinary schistosomiasis (WHO 2006, 2013).

According to WHO guidelines, PZQ should be given once yearly to all school-age children and adults at risk in high-risk communities ( $\geq 50\%$  prevalence of infection by parasitological methods), once every 2 years to all school-age children and adults at risk in moderate-risk communities ( $\geq 10\%$  but  $< 50\%$  prevalence by parasitological methods), and twice during primary schooling age to all school-age children and as a case-directed treatment for adults in low-risk communities ( $< 10\%$  prevalence by parasitological methods). Safety of PZQ is not established in children below 4 years; therefore, these children should be excluded from mass treatment but can be treated on an individual basis by medical personnel. The dosage of PZQ is determined according to the height of children. Possible indicators for monitoring preventive chemotherapy interventions are as follows: prevalence of infection (by parasitological methods), intensity of infection (proportion of heavy-intensity infections), and prevalence of macrohaematuria, microhaematuria, anaemia, or ultrasound-detectable lesions (urinary tract and liver) (WHO 2006).

### **3.9.2 Vaccine Development**

#### **3.9.2.1 The Immediate Need of a Vaccine**

For combating schistosomiasis, chemical and biological molluscicides have proven efficacy, yet may be harmful to the environment (Combes and Cheng 1986). Health and hygiene education and improving sanitary and living conditions are required, yet are not available and are not expected to be available in a near future in most countries where schistosomiasis is endemic (Uttinger et al. 2009, 2011; King 2010). Despite the massive distribution and use of the rather safe, effective, and cost affordable schistosomicide, PZQ, it was given to only 30–40 million patients, while hundreds of millions at large remained without treatment. Chemotherapeutic treatment neither delays nor prevents reinfection, requiring repeated administration, and increases the probability of development of parasite resistance to the drug. Accordingly, a safe, efficacious, and cost-effective vaccine should be available to children in endemic rural areas without any delay, in an aim to interrupt transmission and eliminate schistosomiasis.

#### **3.9.2.2 The Prospects of the Candidate Vaccine Antigens**

During the 1990s of the last century, a number of candidate vaccine antigens were found to be the targets of Th1-/Th2-related immune responses in irradiated cercariae-vaccinated mice (reviewed in El Ridi and Tallima 2013a, b) and residents of endemic countries, such as Egypt and Brazil, that are susceptible or resistant to reinfection after praziquantel treatment (de Jesus et al. 2000; Bergquist et al. 2002; Al-Sherbiny et al. 2003). The selected molecules were purified, characterized, and prepared in a recombinant or multiple antigen peptide (MAP) form. The test

molecules, likely emulsified in Freund's or alum adjuvant, failed to induce the benchmark of 50 % protection in mice in independent trials sponsored by the World Health Organization (Bergquist and Colley 1998; Todd and Colley 2002). IrV5 studies were discontinued. Experiments with triose-phosphate isomerase (TPI) MAP-1 and MAP-2 (Reis et al. 2008) showed that no protection was induced in mice following immunization with TPI together with immunogenic epitopes of glutathione S-transferase, paramyosin, calpain, and Sm23, assembled as DNA, recombinant protein, or MAP constructs (Yang et al. 2000). Yet, immunization of BALB/c mice with a codon-optimized *S. japonicum* TPI construct elicited higher than 50 % reduction in challenge *S. japonicum* worm burden and worm egg counts (Zhu et al. 2010). Glyceraldehyde 3-phosphate dehydrogenase (SG3PDH) in a recombinant, linear peptide, di- and tetrabranching MAP construct, administered to inbred and outbred mice intramuscularly or subcutaneously without adjuvant or emulsified in Freund's, alum, Allison's, or Ribi adjuvant, led to only 10–35 % protection against challenge *S. mansoni* infection [reviewed in El Ridi (1998) and El Ridi and Tallima (2013a, b)]. Trials with the large chain of calpain Sm-80 in a recombinant or DNA vaccine construct were successful, whereby approximately 50 % reductions in worm burdens and worm egg loads were achieved in inbred mice and outbred baboons (Siddiqui et al. 2011). Regarding paramyosin, a good manufacturing practice-ready, pilot-scale process to produce recombinant full-length *S. japonicum* paramyosin, rSj97, was established, and efficacy and safety studies are currently conducted in rodents and large-animal models (Jiz et al. 2008, 2009).

Sm23, a member of the tetraspanin family of molecules located at the host–parasite interface, was used in multiple trials in a recombinant (large extracellular hydrophilic domain) construct in conjunction with alum or Th1-type adjuvants, resulting in limited levels of protection of inbred C57BL/6 mice against subsequent challenge infection (Da'Dara et al. 2003). Vaccination with nucleic acid constructs encoding Sm23 or Sj23 effectively induced parasite-specific IFN- $\gamma$  and Th1-/Th2-related antibody responses yet failed to evoke other critical responses needed for optimal vaccine efficacy (Da'Dara et al. 2002, 2003; Gan et al. 2005; Ganley-Leal et al. 2005). More recently, other members of the tetraspanin family of integral membrane proteins, namely, Sm-TSP-1 and Sm-TSP-2, emulsified in Freund's adjuvant were found to induce significant ( $P < 0.001$ ) levels of protection in CBA/CaH mice against schistosomiasis mansoni (Tran et al. 2006). These findings were not reproduced on using *S. japonicum* tetraspanins (Zhang et al. 2011). However, large amounts of the extracellular domain of Sm-TSP-2 are being now generated at Baylor College of Medicine, ready for phase I clinical studies (Curti 2012), notwithstanding its poor performance (Cai et al. 2008; Zhang et al. 2011; Pearson et al. 2012) and the lack of highly significant protective potential of other integral membranes such as Sm23 (Ganley-Leal et al. 2005), *S. mansoni* glucose transporter SGTP4 (Mahana 2006), and numerous other molecules residing in the tegument of the parasite (Cardoso et al. 2008; Rofatto et al. 2013; Teixeira de Melo et al. 2013).

Of note, glutathione S-transferase of *S. haematobium* progressed to clinical trial phase I using alum as adjuvant; safety and tolerability were documented, but no reports on the efficacy against urinary schistosomiasis are available as yet (Riveau et al. 2012). The fatty acid-binding protein Sm14 also progressed to preclinical and clinical studies (Tendler and Simpson 2008); yet, no information concerning efficacy against infection with *S. mansoni* were reported.

Surprisingly, most if not all schistosomiasis vaccine experiments aimed to achieve preponderance of Th1-related cytokines and antibodies (Da’Dara et al. 2003; Cardoso et al. 2008; Siddiqui et al. 2011; Teixeira de Melo et al. 2013; Wang et al. 2013), while resistance to schistosome reinfection in humans, to whom the vaccine is supposedly dedicated, was previously and recently shown to be dependent on type 2 immune responses (Hagan et al. 1991; Roberts et al. 1993; Fallon et al. 2003; Ganley-Leal et al. 2006; Jiz et al. 2009; Figueiredo et al. 2012; Fitzsimmons et al. 2012).

### 3.9.2.3 The Breakthrough

#### A Plausible Mechanism of Immunity

Schistosomes reside in the bloodstream impervious to immune attacks. If intact parasites were susceptible to killing via antibody-dependent complement activation or cell-mediated cytotoxicity (ADCC), they would not survive a day, least of all a decade, as surface membrane-associated molecules released from dying or dead parasites are readily accessible to the host and are immunogenic (Mahana 2006; Tran et al. 2006). Yet, generated antibodies are not able to access antigens on the apical membrane or the tegument (El Ridi and Tallima 2013a, b and references therein; Migliardo et al. 2014). The immune system effectors may, however, recognize and interact with the “scent” molecules, the ESP of developing and adult worms, resulting into immune cell activation and release of inflammatory and toxic mediators in the vicinity of the parasite. In large vessels, ESP are easily washed away from the parasite. The situation is more difficult for the developing worms in the lung capillaries and liver sinusoids, where the ESP might stagnate, attracting the hunting cells in close proximity to the migrating schistosomula. Accordingly, ESP released by developing schistosomula should be excellent vaccine candidates. Indeed, many of the candidate vaccine molecules are larval ESP (El Ridi and Tallima 2009).

To develop an ESP-based vaccine, it is important to examine the “hunting” team summoned upon parasite invasion (von Lichtenberg et al. 1977). Larval ESP induce predominantly Th1-/Th17-related cytokines and antibody responses in mice and humans during natural infection and in mice following immunization with schistosome antigens and Th1-/Th17-biased adjuvants. Accordingly, circulating monocytes and neutrophils would be readily recruited and activated and certainly contribute to the demise of a proportion of invading larvae. Yet, eosinophils and basophils are not invited to participate in the chase and are actually entirely



excluded (von Lichtenberg et al. 1977). To recruit and activate eosinophils and basophils, it is necessary to use an adjuvant that skews the larval-induced Th1/Th17 immune responses towards the type 2 phenotype (El Ridi and Tallima 2012, 2013a, b and references therein).

### Adjuvant Selection

Our efforts for proper adjuvant choice led to selection of the type 2 immune response-inducing thymic stromal lymphopoietin, TSLP (El Ridi and Tallima 2012), and papain. Four consecutive experiments were set up whereby groups of 5–10 outbred CD1 mice were injected subcutaneously with 200  $\mu$ l Dulbecco's phosphate-buffered saline (D-PBS), pH 7.1, or 50  $\mu$ g papain in 200  $\mu$ l D-PBS, 1 h before whole-body exposure to 125 cercariae of *S. mansoni*. Worm burden was evaluated 6 weeks after infection. For every experiment, papain injection consistently and reproducibly elicited highly significant ( $P < 0.0001$ ) reduction in worm burden of  $70\% \pm 3$  (mean  $\pm$  SD for four independent experiments). The reduction was also highly significant for worm liver ( $P < 0.0001$ ) and small intestine ( $P < 0.001$ ) egg counts but only of approximately 50% (Table 3.1). Papain has been documented to drive ovalbumin immune responses towards the type 2 axis within a few days after injection in mice (Sokol et al. 2008, 2009; Tang et al. 2010), and that was sufficient, without any previous or additional immunization, to lead to highly significant ( $P < 0.0001$ ) protection against schistosomiasis mansoni in every test mouse as compared to controls. These findings gave a sound proof that a large proportion of invading *S. mansoni* larvae would succumb if met upon host invasion with type 2 immune responses.

### A Vaccine Formula

A series of 8 successive experiments led us to define a formula for an efficacious vaccine for schistosomiasis, leading to consistent, reproducible, and highly significant ( $P < 0.0001$ ) reduction in challenge *S. mansoni* worm burden and worm egg load in the liver and small intestine of 62–75% in immunized outbred, akin to man, mice as compared to controls (El Ridi and Tallima 2013c). The formula is as follows: larval ESP mix + type 2 cytokine such as TSLP, IL-25, or IL-33. It is important to examine the different ESP for optimal results. In our hands, a mixture of rSG3PDH and a MAP construct based on a peptide of *S. mansoni* 2-cys peroxiredoxine (TPX MAP Mix) was more protective than a mixture of rSG3PDH and a MAP construct based on a peptide of *S. mansoni* aldolase (ALD MAP Mix) upon immunization of outbred mice in conjunction with TSLP (200 ng/mouse) or papain (10  $\mu$ g/mouse) as adjuvant (El Ridi 2012; El Ridi and Tallima 2013a).

**Table 3.1** Effect of papain administration before infection with *S. mansoni* on parasitological parameters

Mean + SD ( <i>P</i> value; reduction) in mice	Untreated	Papain-treated
Total worm burden	39.9 ± 9.1	13.1 ± 3.2 (<0.0001; 67.16 %)
Male worm burden	20.6 ± 6.2	7.1 ± 2.6 (<0.0001; 65.53 %)
Female worm burden	19.2 ± 4.0	6.0 ± 0.75 (<0.0001; 68.75 %)
Liver egg counts	36,350 ± 7,777	17,187 ± 3,575 (<0.0001; 52.71 %)
Small intestine egg counts	31,035 ± 9,251	15,187 ± 3,835 (0.0002; 51.06 %)

Representative of four independent experiments whereby ten mice/group were subcutaneously injected with 100 µl phosphate-buffered saline, pH 7.1 (PBS) or 50 µg papain in 100 µl PBS one hour before exposure to *S. mansoni* cercariae via whole body exposure. Parasitological parameters were evaluated six weeks after infection, and data analyzed using Student's *t*-test

### A Proof of Concept Leads to an Adjuvant-Free Vaccine Formulation

Immunization of outbred mice with schistosome antigens, which are both ESP and type 2 immune response inducing, consistently and reproducibly elicited highly significant ( $P < 0.0001$ ) reduction (60–75 %) of challenge *S. mansoni* worm burden and worm egg load in the liver and small intestine as compared to unimmunized mice. It is, thus, fortunate that we developed an adjuvant-free Th2 immune response-based vaccine effective in outbred mice, since it is destined to the outbred human population, where adjuvant use remains a considerable challenge and where immunological correlates of resistance to the 3 species of schistosomes are associated with type 2 responses (El Ridi et al. 2014).

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