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Biology of the schistosome lung-stage schistosomulum

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SUMMARY

Past and more recent research has examined the ultrastructure, metabolism, cell biology, genomics and post-genomics of schistosome schistosomula. These areas are considered and discussed in this review with particular emphasis on (1) the early migration phases through the host, (2) interaction of the host immune response with the parasite surface, (3) glucose uptake mechanisms, and (4) defining the transcriptional profiles of lung-stage schistosomula compared with other developmental stages using microarrays. The microarray profiling studies suggest caution is required when considering the use of schistosomes obtained by *in vitro* means for molecular or biochemical studies.

Keywords

schistosome; lung schistosomula; immune evasion; *in vitro* model

INTRODUCTION

Penetration of the skin of the definitive host by a schistosome results in the cercaria losing its tail, triggering development into the schistosomulum, with the whole process being known as the cercarial/schistosomulum transformation (Stirewalt and Dorsey, 1974; He *et al.* 2005). This morphological transformation is accompanied by dramatic changes in the physiology and biochemistry of the larva (Stirewalt, 1974). Contraction and extension of the schistosomulum and the release of enzymes from its pre- and post-acetabular glands causes extensive tissue damage to all host structures traversed, until the larva breaches a blood vessel wall and enters a blood vessel. The timing of these early skin-schistosomulum stage events varies in schistosome species. Ninety percent of *Schistosoma mansoni* and *S. haematobium* parasites still remain in the host epidermis after 24 h while within the same period a similar number of *S. japonicum* schistosomula will have already reached the dermis or dermal vessels of the host (He *et al.* 2002).

The schistosomula then travel via the pulmonary artery to the lungs where they are located intra-vascularly and are now referred to as lung schistosomula (Wilson and Coulson, 1989; Dean and Mangold, 1992). Again, timing of the initial arrival and period within the lung varies between species, with peak appearance being on day 5–6 post-infection for *S. mansoni* with some parasites detected up to day 20, although it is not clear at this time-point whether the parasites have just arrived or have become trapped in the lungs. *S. japonicum* schistosomula peak in the lungs at day 3 after penetration (Gui *et al.* 1995). After exiting the lungs, schistosomula re-enter the venous circulation (Gui *et al.* 1995). The pre-adult

schistosome can then reside in the liver for up to 15 days post-penetration (Mitchell *et al.* 1991). In the liver, the parasites grow and develop for 8-10 days before forming pairs, and finally they lodge in the portal and mesenteric vessels of the gut from day 11 (for *S. japonicum*) (He, 1993).

IMMUNOBIOLOGY OF THE LUNG-STAGE SCHISTOSOMULUM

After penetrating the definitive host, significant morphological and biochemical changes occur to the developing schistosomulum, including the modification of surface or secreted antigens that may otherwise stimulate an immune response (Mountford and Harrop, 1998). Skin-stage schistosomula are susceptible to humoral and cellular immune responses, but rapidly develop resistance to host immunity (Dean, 1977; El-Ridi *et al.* 2003). This is illustrated by the relative sensitivity of skin schistosomula to both complement-mediated killing and antibody-dependent cellular cytotoxicity, whereas the lung schistosomula and adults become increasingly resistant to both of these immunological defences (McLaren, 1980; Lawson *et al.* 1993; Parizade *et al.* 1994). This increasing degree of resistance may be linked to the shedding of the cercarial membrane and formation of the heptalaminate surface membrane several hours after penetration (Hockley and McLaren, 1973; Gobert *et al.* 2003).

Immune evasion was originally attributed to antigen masking but resistance of lung-stage schistosomula to immune effector mechanisms was reported regardless of host antigen adsorption (Dean, 1977). This implied structural or biochemical modifications of the membrane surface, preventing binding of antibody or reducing antigen expression (El-Ridi *et al.* 2003). It has been suggested that the lung-stage schistosomulum protectively confines antigenic molecules in specific, lipid-rich sites of the outer surface membrane and that, in addition to this physical barrier, biochemical and immunological barriers must exist to protect the parasite from the host immune response (Parizade *et al.* 1994; El-Ridi *et al.* 2003). This may reflect the presence of lipid-rafts on the schistosome surface membrane that effectively allow compartmentalization of regions, as an immunological camouflage (Grossmann *et al.* 2006). The importance of the outer membrane of the schistosomulum in the avoidance of immunological attack was examined by Tallima and El-Ridi (2005) who selectively depleted the cholesterol content of *S. mansoni* schistosomula, which led to binding of schistosome-specific antibodies, as shown by immunofluorescence. However, a parallel experiment in the same study, using *S. haematobium* schistosomula, failed to enhance antibody binding, strongly suggesting that the lipid composition differs between the two species.

In contrast to the evidence for their relative immune insusceptibility to unattenuated cercariae in mice, lung schistosomula were found to be the principal target of immune mechanisms in the rat model, in response to either radiation-attenuated (RA) or unattenuated cercariae prior to exposure (Ford *et al.* 1984; Knopf *et al.* 1986). Similarly, elimination of schistosomula from the lungs of mice after vaccination with RA cercariae (Wilson and Coulson, 1989; Hewitson *et al.* 2005) was found to be proportional to the time the parasites spent in the lungs (Dean and Mangold, 1992). Moreover, antigens obtained from lung-stage larvae are apparently more efficient stimulators of lymphocyte proliferation and secretion of Th1 cytokines than those from cercariae and skin-stage larvae (Mountford *et al.* 1995). Overall, until recently, there was a paucity of information regarding the lung-stage schistosomulum and its tegumental surface and secreted molecules, despite the acknowledged acceptance of antigens derived from this stage as being potentially important vaccine candidates (Mountford *et al.* 1995; Gobert *et al.* 1997; Mountford and Harrop, 1998; Harrop *et al.* 1999).

THE LUNG-STAGE SCHISTOSOMULUM AND IMMUNE EVASION

Although the immunobiology of the lung schistosomulum remains a relatively poorly investigated topic, several interpretations regarding its defences against the host response can be made from morphological observations. One critical morphological difference is the complete absence of the highly immunogenic cercarial glycocalyx on the surface of the lung-stage schistosomulum, which is retained for some time in the newly transformed schistosomulum as it migrates through the skin (Agoston *et al.* 2001). However, it seems that lung schistosomula do activate complement (Santoro *et al.* 1979) and bind antibodies at their surface (regardless of the lack of the glycocalyx); they must therefore resist immune damage by some other means, perhaps related to the formation of the heptalaminate membrane (Lawson *et al.* 1993; Gobert *et al.* 2003). Indeed, early studies suggested that the insusceptibility of post-skin-stage schistosomula to antibody-dependent killing resulted at least in part from an intrinsic structural change in the tegument of the parasite that could not be caused solely by the masking of parasite antigens by acquired host molecules (Moser *et al.* 1980). A similar pattern of increased insusceptibility to host immune attack by lung schistosomula compared with skin forms was demonstrated in response to nitric oxide (NO), an active component of cell-mediated killing (Ahmed *et al.* 1997). The mechanism of NO toxicity involves direct interaction with metabolic processes via the disruption of key enzymes. Loss of susceptibility to NO in schistosomes may be linked to the shift from aerobic to anaerobic metabolism and conversion to a less metabolically active state in lung schistosomula, and may be considered a passive defence strategy rather than active immune evasion (Ahmed *et al.* 1997).

Chai *et al.* (2006) described detailed ultrastructural analysis of the tegument of the lung schistosomulum which revealed no evidence of tegument sloughing but a dynamic state of tegumental remodelling and turnover. The suggested association of the membranous contents of elongated and membranous bodies becoming incorporated into the apical membrane of the tegument appear likely, as originally suggested by Hockley and McLaren (1973) from their observations of adult *S. mansoni*. Although ultrastructural analysis of the lung-stage schistosomulum can provide some insights regarding the ability of this life-cycle stage to endure immune attack through gross mechanisms such as tegument thickening or shedding, molecular/biochemical techniques must be used to address the question of why the lungs are the principal site of worm elimination. Through profiling the spectrum of proteins and glycoconjugates present at the host/parasite interface a full appreciation of the molecular defences of schistosomes can be made.

RECOVERY AND COMPARATIVE STUDY OF SCHISTOSOMULA OBTAINED BY *IN VIVO* AND *IN VITRO* PROCEDURES

Schistosomula may be recovered directly from the lungs by mincing lung tissue from the mammalian host at various times post-infection (Clegg, 1965; Gui *et al.* 1995). We will refer to these *in vivo* isolated schistosomula as LS (lung schistosomula). As an alternative, mechanically transformed schistosomula (see below) have been maintained in culture *in vitro* for different times to provide all the mammalian host stages, including lung-stage schistosomula, and even paired, mature adult males and females (Basch, 1981; Basch and Basch, 1982). We will refer to these *in vitro* isolated and maintained schistosomula as MT (mechanically transformed schistosomula). MT are now commonly used in studies of ultrastructure, genetics and gene manipulation, development, behaviour, metabolic activity, biochemistry, molecular biology, immunology and in vaccine development (Cousin *et al.* 1981; Salafsky *et al.* 1988; Marikovsky *et al.* 1990; Harrop and Wilson, 1993; Harrop *et al.* 1999; Gold and Fletcher, 2000; Loukas *et al.* 2001; Correnti *et al.* 2005). However, there is often a lack of standardization associated with the various MT methods for obtaining

different intra-mammalian schistosome stages, with culturing durations or conditions being varied and sometimes arbitrary (Harrop and Wilson, 1993; Lawson *et al.* 1993; Curwen *et al.* 2004). While there is evidence that MT are structurally similar to their LS counterparts (Chai *et al.* 2006), the comparative molecular biology and biochemistry of schistosomes obtained by the two approaches is poorly understood (Brink *et al.* 1977; Samuelson *et al.* 1980; Cousin *et al.* 1981; Gold and Fletcher, 2000).

Commonly used *in vitro* cercarial transformation techniques include the mechanical detachment of the tail (by centrifugation, vortexing or pressure shearing), penetration of cercariae through isolated skin, and incubation of cercariae in sera, chemicals and/or media varying in glucose concentration, osmolarity, or amino acid and lipid content (Ramalho-Pinto *et al.* 1974, 1975; Brink *et al.* 1977; Cousin *et al.* 1981; Salafsky *et al.* 1988; Horemans *et al.* 1992). However, comprehensive comparisons of schistosomula obtained by these various procedures, and with parasites obtained directly from the lungs are lacking. MT are determined to be of a particular developmental stage by gross morphology and/or culture duration (Basch, 1981). Previous reports that have used MT in studies of protein/antigen release or antigenicity, involved culturing for 3 h (Lawson *et al.* 1993), 7 days (Harrop and Wilson, 1993), 8 days (Curwen *et al.* 2004) and up to 11 days (Mountford *et al.* 1995). This variation in protocol exemplifies the arbitrary nature of culture duration for MT schistosomes; while in addition it is well known that without the presence of erythrocytes in culture media, progression of the parasites to the liver stage of development was prevented (Basch, 1981).

Cousin *et al.* (1981) compared the ultrastructure of MT with that of *in vivo* skin-phase schistosomula and concluded that their developmental patterns achieved the same end-point but occurred at different rates. Samuelson *et al.* (1980) also found that the gross morphologies, determined by scanning electron microscopy of MT and LS were similar. However, *S. mansoni* MT do become more elongate with time in culture albeit not to the same extent as LS (Cousin *et al.* 1981).

THE TEGUMENT OF THE LUNG-STAGE SCHISTOSOMULUM AND GLUCOSE UPTAKE

Whereas cercariae derive energy only from endogenous glycogen reserves, adult schistosomes are dependent primarily on glucose absorbed from the blood of the mammalian host (Bruce *et al.* 1969; Halton, 1997). Glucose and other small molecular weight solutes are transported across the tegument, with adult schistosomes reportedly consuming their dry weight in glucose every 5 h, using mechanisms that include the use of glucose transporter proteins (SGTPs) (Skelly *et al.* 1998; Brouwers *et al.* 1999). The cercaria/schistosomulum transformation includes a switch from aerobic to anaerobic energy metabolism, loss of the glycocalyx and complete reconstruction of the tegumental membranes (Skelly and Shoemaker, 2000). The glucose transporter proteins SGTP1 and SGTP4 have been localized to the tegument of adult parasites and freshly obtained *in vitro* mechanically transformed schistosomula, but SGTP4 is absent in cercariae and the other extra-mammalian forms, all of which lack the heptalaminate membrane of the intra-mammalian parasites (Jiang *et al.* 1996; Skelly and Shoemaker, 1996). The presence of SGTPs has not been reported in schistosomula obtained directly from the lungs of mammalian hosts and it is unknown how schistosomula in the lung supplement their depleted internal stores of glycogen (Skelly and Shoemaker, 1995; El-Ridi *et al.* 2003).

GENE EXPRESSION STUDIES

Fundamental differences in gene expression between schistosomula obtained by *in vivo* and *in vitro* procedures were reported in the recent microarray study by Chai *et al.* (2006). A 19 221 feature oligo-nucleotide microarray was used to compare the transcription profiles of *S. japonicum* schistosomula, isolated directly from the lungs of mice 3 days post-infection, with mechanically transformed schistosomula maintained in culture for the same period. Differential gene expression was correlated with biological function relating specifically to components involved in schistosome immune evasion and nutrition. The most striking aspect of the analysis was the large number (6662) of genes differentially expressed between the two forms, indicating major biochemical and physiological differences between the two schistosomula types. This suggests that data obtained from schistosomula cultured *in vitro* may present an under- or over-estimate of gene expression compared with parasites obtained directly from the lungs.

This potential anomaly is illustrated in Table 1 where a comparison has been made of the most abundant proteins from mechanically transformed/8-day *in vitro* cultured schistosomula of *S. mansoni* (Curwen *et al.* 2004) with transcription levels of mechanically transformed/cultured versus *in vivo* lung-recovered schistosomula of *S. japonicum* (Chai *et al.* 2006). Although comparison of protein and relative transcription levels are not directly comparable and the fact the 2 different schistosome species were examined in the 2 studies, the clear differential gene expression that was demonstrated for MT and LS of *S. japonicum* (Chai *et al.* 2006) suggests that the protein abundance findings of Curwen *et al.* (2004) may require normalization. While it is important to note that MT can be introduced to the mammalian stage via intravenous injection and progress normally (Sher *et al.* 1984), it is not clear whether the parasite dramatically alters its gene expression profile to adapt to the *in vivo* situation.

Similarly, disparity in gene expression must be considered in the microarray results of Dillon *et al.* (2006) who compared MT of *S. mansoni* with liver stage and adult parasites. Again when correlating the findings of Dillon *et al.* (2006) with those of Chai *et al.* (2006), potential over- or under-estimation of the expression of gene products in MT parasites is apparent (Table 2). Full validation of previous studies using *in vitro*-obtained *S. mansoni* schistosomula cannot be made until a comparison of *S. mansoni* MT and LS is undertaken. We acknowledge that gene expression differences will occur between the two closely related schistosome species, but the findings of Chai *et al.* (2006) do highlight the possible limitations of using MT for study, a concern raised also by others (Contreras *et al.* 1998).

CONCLUSIONS

Further mining of the microarray data from the study of Chai *et al.* (2006) will undoubtedly reveal many more genes or clustered sequences of basic biological interest, elucidation of the function of their expressed products, and an assessment of their potential as vaccine or drug targets.

The differential gene expression profiles of lung schistosomula and those obtained by mechanical transformation/*in vitro* culture suggest that the use of the latter may potentially produce under- or over-estimations of actual parasite gene expression and associated physiology/biochemistry. Schistosomes produced by *in vitro* methods are utilized on the premise that schistosome development can be simulated independently of the host environment, and that observed morphological similarities confer overall equivalence of *in vitro* with *in vivo* forms. However, it seems likely that factors in the host environment encountered by mammalian stage schistosomes within the host are reflected at the

transcriptome level. This would suggest that studies using *in vitro*-derived schistosomula are useful when examining gross morphology or pre-determined patterns of schistosome development, but the use of schistosomes obtained by *in vitro* means for molecular or biochemical studies should be carefully considered.

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REFERENCES

- Agoston K, Kerekgyarto J, Hajko J, Batta G, Lefebvre D, Kamerling J, Vliegenthart JFG. Synthesis of fragments of the glycocalyx glycan of the parasite *Schistosoma mansoni*. *Chemistry - A European Journal*. 2001; 8:151–161.
- Ahmed SF, Oswald IP, Caspar P, Hieny S, Keefer L, Sher A, James SL. Developmental differences determine larval susceptibility to nitric oxide-mediated killing in a murine model of vaccination against *Schistosoma mansoni*. *Infection and Immunity*. 1997; 65:219–226. [PubMed: 8975915]
- Basch PF. Cultivation of *Schistosoma mansoni* *in vitro*. 1. Establishment of cultures from cercariae and development until pairing. *Journal of Parasitology*. 1981; 67:179–185. [PubMed: 7241277]
- Basch PF, Basch N. *Schistosoma mansoni*: scanning electron microscopy of schistosomula, adults and eggs grown *in vitro*. *Parasitology*. 1982; 85:333–338. [PubMed: 7145475]
- Brink LH, McLaren DJ, Smithers SR. *Schistosoma mansoni*: a comparative study of artificially transformed schistosomula and schistosomula recovered after cercarial penetration of isolated skin. *Parasitology*. 1977; 74:73–86. [PubMed: 320543]
- Brouwers JFHM, Skelly PJ, VanGolde LMG, Tielens GM. Studies on phospholipid turnover argue against sloughing of tegumental membranes in adult *Schistosoma mansoni*. *Parasitology*. 1999; 119:287–294. [PubMed: 10503254]
- Bruce JJ, Weis EM, Stirewalt MA, Lincicome DR. *Schistosoma mansoni*: glycogen content and utilization of glucose, pyruvate, glutamate, and citric acid cycle intermediates by cercariae and schistosomules. *Experimental Parasitology*. 1969; 26:29–40. [PubMed: 5362313]
- Chai M, McManus DP, McInnes R, Moertel LP, Tran M, Loukas A, Jones MK, Gobert GN. Transcriptome profiling of lung schistosomula, *in vitro* cultured schistosomula and the adult *Schistosoma japonicum*. *Cellular and Molecular Life Sciences*. 2006; 63:919–929. [PubMed: 16570121]
- Clegg JA. *In vitro* cultivation of *Schistosoma mansoni*. *Experimental Parasitology*. 1965; 16:133–147. [PubMed: 14281210]
- Contreras VT, De Lima AR, Zorrilla G. *Trypanosoma cruzi*: maintenance in culture modify gene and antigenic expression of metacyclic trypomastigotes. *Memorias do Instituto Oswaldo Cruz*. 1998; 93:753–760. [PubMed: 9921298]
- Correnti JM, Brindley PJ, Pearce EJ. Long-term suppression of cathepsin B levels by RNA interference retards schistosome growth. *Molecular and Biochemical Parasitology*. 2005; 143:209–215. [PubMed: 16076506]
- Cousin CE, Stirewalt MA, Dorsey CH. *Schistosoma mansoni*: ultrastructure of early transformation of skin- and shear-pressure-derived schistosomules. *Experimental Parasitology*. 1981; 51:341–365. [PubMed: 7227486]
- Curwen RS, Ashton PD, Johnston DA, Wilson RA. The *Schistosoma mansoni* soluble proteome: a comparison across four life-cycle stages. *Molecular and Biochemical Parasitology*. 2004; 138:57–66. [PubMed: 15500916]
- Dean DA. Decreased binding of cytotoxic antibody by developing *Schistosoma mansoni*. Evidence for a surface change independent of host antigen adsorption and membrane turnover. *The Journal of Parasitology*. 1977; 63:418–426. [PubMed: 325186]

- Dean DA, Mangold BL. Evidence that both normal and immune elimination of *Schistosoma mansoni* take place at the lung stage of migration prior to parasite death. *American Journal of Tropical Medicine and Hygiene*. 1992; 47:238–248. [PubMed: 1503191]
- Dillon GP, Feltwell T, Skelton JP, Ashton PD, Coulson PS, Quail MA, Nikolaidou-Katsaridou N, Wilson RA, Ivens AC. Microarray analysis identifies genes preferentially expressed in the lung schistosomulum of *Schistosoma mansoni*. *International Journal for Parasitology*. 2006; 36:1–8. [PubMed: 16359678]
- El-Ridi R, Mohamed SH, Tallima H. Incubation of *Schistosoma mansoni* lung-stage schistosomula in corn oil exposes their surface membrane antigenic specificities. *The Journal of Parasitology*. 2003; 89:1064–1067. [PubMed: 14627160]
- Ford MJ, Bickle QD, Taylor MG, Andrews BJ. Passive transfer of resistance and the site of immune-dependent elimination of the challenge infection in rats vaccinated with highly irradiated cercariae of *Schistosoma mansoni*. *Parasitology*. 1984; 89:461–482. [PubMed: 6514379]
- Gobert GN, Stenzel DJ, Jones MK, Allen DE, McManus DP. *Schistosoma japonicum*: immunolocalization of paramyosin during development. *Parasitology*. 1997; 114:45–52. [PubMed: 9011073]
- Gobert GN, Stenzel DJ, McManus DP, Jones MK. The ultrastructural architecture of the adult *Schistosoma japonicum* tegument. *International Journal for Parasitology*. 2003; 33:1561–1575. [PubMed: 14636672]
- Gold D, Fletcher E. Influence of mechanical tail-detachment techniques of schistosome cercariae on the production, viability, and infectivity of resultant schistosomula: a comparative study. *Parasitology Research*. 2000; 86:570–572. [PubMed: 10935908]
- Grossmann G, Opekarova M, Novakova L, Stolz J, Tanner W. Lipid raft-based membrane compartmentation of a plant transport protein expressed in *Saccharomyces cerevisiae*. *Eukaryotic Cell*. 2006; 5:945–953. [PubMed: 16757742]
- Gui M, Kusel JR, Shi RE, Ruppel A. *Schistosoma japonicum* and *S. mansoni*: comparison of larval migration patterns in mice. *Journal of Helminthology*. 1995; 69:19–25. [PubMed: 7622787]
- Halton DW. Nutritional adaptations to parasitism within the platyhelminthes. *International Journal for Parasitology*. 1997; 27:693–704. [PubMed: 9229252]
- Harrop R, Coulson PS, Wilson RA. Characterization, cloning and immunogenicity of antigens released by lung-stage larvae of *Schistosoma mansoni*. *Parasitology*. 1999; 118:583–594. [PubMed: 10406037]
- Harrop R, Wilson RA. Protein synthesis and release by cultured schistosomula of *Schistosoma mansoni*. *Parasitology*. 1993; 107:265–274. [PubMed: 8233590]
- He YX. Biology of *Schistosoma japonicum* from cercaria penetrating into host skin to producing egg. *Chinese Medical Journal*. 1993; 106:576–583. [PubMed: 8222905]
- He YX, Chen L, Ramaswamy K. *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*: early events associated with penetration and migration of schistosomula through human skin. *Experimental Parasitology*. 2002; 102:99–108. [PubMed: 12706745]
- He YX, Salafsky B, Ramaswamy K. Comparison of skin invasion among three major species of *Schistosoma*. *Trends in Parasitology*. 2005; 21:201–203. [PubMed: 15837605]
- Hewitson JP, Hamblin PA, Mountford AP. Immunity induced by the radiation-attenuated schistosome vaccine. *Parasite Immunology*. 2005; 27:271–280. [PubMed: 16138848]
- Hockley D, McLaren DJ. *Schistosoma mansoni*: changes in the outer membrane of the tegument during development from cercaria to adult worm. *International Journal for Parasitology*. 1973; 3:13–25. [PubMed: 4687430]
- Horemans AMC, Tielens A, van den Bergh SG. The reversible effect of glucose on the energy metabolism of *Schistosoma mansoni* cercariae and schistosomula. *Molecular and Biochemical Parasitology*. 1992; 51:73–80. [PubMed: 1565140]
- Jiang J, Skelly PJ, Shoemaker CB, Caulfield JP. *Schistosoma mansoni*: the glucose transport protein SGTP4 is present in tegumental multilamellar bodies, discoid bodies, and the surface lipid bilayers. *Experimental Parasitology*. 1996; 82:201–210. [PubMed: 8617347]

- Knopf PM, Cioli D, Mangold BL, Dean DA. Migration of *Schistosoma mansoni* in normal and passively immunized laboratory rats. *American Journal of Tropical Medicine and Hygiene*. 1986; 35:1173–1184. [PubMed: 3098124]
- Lawson BWL, Bickle QD, Taylor MG. Mechanisms involved in the loss of antibody-mediated adherence of macrophages to lung-stage schistosomula of *Schistosoma mansoni* *in vitro*. *Parasitology*. 1993; 106:463–469. [PubMed: 8341582]
- Loukas A, Jones MK, King LT, Brindley PJ, McManus DP. Receptor for Fc on the surface of schistosomes. *Infection and Immunity*. 2001; 69:3646–3651. [PubMed: 11349025]
- Marikovskiy M, Parizade M, Arnon R. Complement regulation on the surface of cultured schistosomula and adult worms of *Schistosoma mansoni*. *European Journal of Immunology*. 1990; 20:221–227. [PubMed: 2307175]
- McLaren, DJ. *Schistosoma mansoni*: the parasite surface in relation to host immunity. In: Brown, KN., editor. *Tropical Medicine Research Studies Series*. Vol. 1. Research Studies Press; London: 1980. p. 229
- Mitchell GF, Tiu WU, Garcia EG. Infection characteristics of *Schistosoma japonicum* in mice and relevance to the assessment of schistosome vaccines. *Advances in Parasitology*. 1991; 30:167–200. [PubMed: 1906234]
- Moser G, Wassom DL, Sher A. Studies of the antibody-dependent killing of schistosomula of *Schistosoma mansoni* employing haptenic target antigens. I. Evidence that the loss in susceptibility to immune damage undergone by developing schistosomula involves a change unrelated to the masking of parasite antigens by host molecules. *Journal of Experimental Medicine*. 1980; 152:41–53. [PubMed: 7400756]
- Mountford AP, Harrop R. Vaccination against schistosomiasis: the case for lung-stage antigens. *Parasitology Today*. 1998; 14:109–114. [PubMed: 17040717]
- Mountford AP, Harrop R, Wilson A. Antigens derived from lung-stage larvae of *Schistosoma mansoni* are efficient stimulators of proliferation and gamma interferon secretion by lymphocytes from mice vaccinated with attenuated larvae. *Infection and Immunity*. 1995; 63:1980–1986. [PubMed: 7729911]
- Parizade M, Arnon R, Lachmann PJ, Fishelson Z. Functional and antigenic similarities between a 94-kD protein of *Schistosoma mansoni* (SCIP-1) and human CD59. *Journal of Experimental Medicine*. 1994; 179:1625–1636. [PubMed: 7513011]
- Ramalho-Pinto FJ, Gazzinelli G, Howells RE, Mota-Santos TA, Figueiredo EA, Pellegrino J. *Schistosoma mansoni*: a defined system for the step-wise transformation of the cercaria to schistosomule. *Experimental Parasitology*. 1974; 36:360–372. [PubMed: 4139038]
- Ramalho-Pinto FJ, Gazzinelli G, Howells RE, Pellegrino J. Factors affecting surface changes in intact cercariae and cercarial bodies of *Schistosoma mansoni*. *Parasitology*. 1975; 71:19–25. [PubMed: 241040]
- Salafsky B, Fusco AC, Whitley K, Nowicki D, Ellenberger B. *Schistosoma mansoni*: Analysis of cercarial transformation methods. *Experimental Parasitology*. 1988; 67:116–127. [PubMed: 2458958]
- Samuelson JC, Caulfield JP, David JR. *Schistosoma mansoni*: post-transformational surface changes in schistosomula grown *in vitro* and in mice. *Experimental Parasitology*. 1980; 50:369–383. [PubMed: 7428913]
- Santoro F, Lachmann PJ, Capron A, Capron M. Activation of complement by *Schistosoma mansoni* schistosomula: killing of parasites by the alternative pathway and requirement of IgG for classical pathway activation. *Journal of Immunology*. 1979; 123:1551–1557.
- Sher A, Sacks DL, Simpson AJ, Singer A. Dichotomy in the tissue origin of schistosome acquired class I and class II major histocompatibility complex antigens. *Journal of Experimental Medicine*. 1984; 159:952–957. [PubMed: 6366108]
- Skelly PJ, Shoemaker CB. A molecular genetic study of the variations in metabolic function during schistosome development. *Memorias do Instituto Oswaldo Cruz*. 1995; 90:281–284. [PubMed: 8531672]

- Skelly PJ, Shoemaker CB. Rapid appearance and asymmetric distribution of glucose transporter SGTP4 at the apical surface of intramammalian-stage *Schistosoma mansoni*. Proceedings of the National Academy of Sciences, USA. 1996; 93:3642–3646.
- Skelly PJ, Shoemaker CB. Induction cues for tegument formation during the transformation of *Schistosoma mansoni* cercariae. International Journal for Parasitology. 2000; 30:625–631. [PubMed: 10779576]
- Skelly PJ, Tielens AGM, Shoemaker CB. Glucose transport and metabolism in mammalian-stage schistosomes. Parasitology Today. 1998; 14:402–406. [PubMed: 17040830]
- Stirewalt MA. *Schistosoma mansoni*: cercaria to schistosomule. Advances in Parasitology. 1974; 12:115–182. [PubMed: 4141581]
- Stirewalt MA, Dorsey CH. *Schistosoma mansoni*: cercarial penetration of host epidermis at the ultrastructural level. Experimental Parasitology. 1974; 35:1–15. [PubMed: 4815016]
- Tallima H, El Ridi R. Methyl-beta-Cyclodextrin treatment and filipin staining reveal the role of cholesterol in surface membrane antigen sequestration of *Schistosoma mansoni* and *S. haematobium* lung-stage larvae. Journal of Parasitology. 2005; 91:720–725. [PubMed: 16108578]
- Wilson RA, Coulson PS. Lung-phase immunity to schistosomes: a new perspective on an old problem. Parasitology Today. 1989; 5:274–278. [PubMed: 15463231]

Table 1

Correlation of the most abundant proteins (genes) from mechanically transformed and cultured schistosomula (MT) with the relative transcription levels of MT with schistosomula obtained directly from the lungs (LS)

Protein name	Accession no.	Spot number ^a	Probe no.	Annotation	Fold change (Direction)	P-value	Enriched in LS
14-3-3	Q9U491	20, 32, 39					
14-3-3 hom 1	Q26540	2	TC7064	Similar to 14-3-3 protein homolog 1	5.06 (LS)	1.32E-07	✓
Actin	P54470/1	6, 10	Contig8822 Contig8895	<i>S. japonicum</i> actin <i>S. japonicum</i> actin	2.64 (MT) 4.92 (MT)	3.03E-23 1.39E-16	
Aldolase	P53442	29					
ATP:GK	P16641	38					
Calreticulin	Q06814	27					
Cyclophilin	Q26565	1	TC16946	Cyclophilin B	3.49 (LS)	3.96E-20	✓
Enolase	Q27877	11	Contig4432 Contig5939 TC16782	<i>S. japonicum</i> enolase <i>Homo sapiens</i> enolase 1 Enolase (2-phospho-D- glycerate hydro-lyase)	12.8 (LS) 2.43 (LS) 48.48 (LS)	8.97E-15 1.23E-20 0	✓
			Contig4914 Contig8721	<i>S. japonicum</i> enolase <i>S. japonicum</i> enolase	2.71 (MT) 3.46 (MT)	6.84E-07 1.13E-11	
FaBP	P29498	21	Contig1965	<i>S. japonicum</i> FaBP	1.71 (LS)	8.62E-21	✓
GAPDH	P20287	15	Contig8536 Contig8573	Blood fluke GAPDH Blood fluke GAPDH	4.34 (MT) 11.74 (MT)	4.17E-12 0	
GST26	P15964	24					
GST28	P09472	17, 19, 30	TC10486	Glutathione S-transferase 28 kDa	2.16 (LS)	3.98E-09	✓
HSP86	Q26582	23					
Sm21.7	P32070	5	Contig5555 Contig8428	<i>S. japonicum</i> 21.7 kDa antigen <i>S. japonicum</i> 21.7 kDa antigen	1.75 (MT) 4.27 (MT)	1.18E-09 0	
SME16	Q07167	28					
SOD	Q01137	9	TC10844	Cu/Zn-superoxide dismutase [<i>S. mansoni</i>]	6.83 (LS)	1.52E-26	✓
Thioredoxin	18874552	3, 7	Contig719 TC14049	<i>S. japonicum</i> thioredoxin Thioredoxin peroxidase 1 [<i>S. mansoni</i>]	2.43 (LS) 2.95 (LS)	2.42E-43 1.69E-04	✓

Protein name	Accession no.	Spot number ^a	Probe no.	Annotation	Fold change (Direction)	P-value	Enriched in LS
			TC15328	Thioredoxin-related 32 kDa protein	20.96 (LS)	0	
			Contig5222	<i>S. japonicum</i> thioredoxin	2.46 (MT)	1.17E-07	
TPI	P48501	14	Contig6423	<i>S. japonicum</i> triosephosphate isomerase	6.71 (MT)	1.94E-10	

2D electrophoresis and protein sequencing data from Curwen *et al.* (2004), using mechanically transformed *S. mansoni* schistosomula.

Microarray data from Chai *et al.* (2006), comparing mechanically transformed/cultured schistosomula of *S. japonicum* with lung stage schistosomula isolated directly from the lungs.

(Potential under-estimations of protein abundance by using MT are highlighted; while potential over-estimations of protein abundance by using LS are not highlighted.)

^aSpot number on 2D gel.

Table 2

Comparison of the most abundant proteins from mechanically transformed *in vitro*-cultured schistosomula and their relative transcription levels compared with schistosomula obtained directly from the lungs

Contig	Putative product	Microarray probe no.	Annotation	Fold change (Direction)	P-value	Enriched in LS
<i>adult>liver>"lung"</i> ^a						
Sm01393	Cathepsin L	Contig5914	<i>Haemonchus contortus</i> cathepsin L	3.79 (LS)	9.84E-31	✓
Sm03944	Serine protease	TC11743	Putative serine protease-like protein <i>Oryza sativa</i> partial (13%)	4.83 (MT)	3.50E-15	
Sm13110	Cathepsin B1	Contig8492	<i>S. japonicum</i> mRNA for cathepsin B	3.22 (MT)	2.09E-11	
Sm01772	isotype 1 precursor					
Sm12907	Cathepsin B1	Contig8839	<i>S. japonicum</i> cathepsin B endopeptidase	3.64 (MT)	1.91E-12	
	isotype 2 precursor					
Sm12892	LGG	Contig5650	<i>S. japonicum</i> LGG	26.56 (MT)	0	
<i>"lung">liver>adult</i> ^b						
Sm00493	Lactate de-hydrogenase	Contig8557	<i>S. japonicum</i> lactate dehydrogenase-like protein	2.97 (MT)	4.98E-14	
Sm03987	Annexin	Contig8017	<i>S. mansoni</i> annexin	1.34 (MT)	7.68E-04	
Sm12876	SM22.6	Contig8689	<i>S. japonicum</i> 22.6 kDa membrane-associated antigen	6.2 (MT)	2.55E-05	
Sm12939	Tetraspanin 3	TC7927	Tegument antigen SMA22.6 [Blood fluke], partial (33%)	11.37 (MT)	0	
Sm02451	Calpain	TC17049	Calpain [Blood fluke]	4.56 (MT)	8.37E-17	
Sm12462	SGTP4	Contig4074	<i>S. mansoni</i> glucose transporter protein (GTP4)	4.75 (MT)	1.72E-17	
Sm00285	Gynecophoral canal protein	Contig7087	<i>S. japonicum</i> gynecophoral canal protein	1.83 (LS)	2.17E-05	✓
		Contig6643	<i>S. japonicum</i> gynecophoral canal protein mRNA	4.5 (MT)	1n79E-40	
Sm11776	Glycogen branching enzyme	TC17880	1.4-alpha-glucan branching enzyme (Glycogen branching enzyme) partial (35%)	5.73 (MT)	1.35E-20	
Protein analysis data from Dillon <i>et al.</i> (2006), using mechanically transformed/cultured <i>S. mansoni</i> schistosomula						
			Microarray data from Chai <i>et al.</i> (2006), comparing mechanically transformed/cultured schistosomula of <i>S. japonicum</i> with lung stage schistosomula			

(Relative over-expressed genes in schistosomula obtained from the lungs are highlighted in grey, while under-expressed genes are not highlighted.)

^a Highest relative gene expression in adult stage, then liver stage and lowest relative gene expression in 'lung' stage.

Highest relative gene expression in 'lung' stage, then liver stage and lowest relative gene expression in adult stage. LS, schistosomula isolated from lungs; MT, mechanically transformed and cultured schistosomula.