Rhesus macaques self-curing from a schistosome infection can display complete immunity to challenge

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The rhesus macaque provides a unique model of acquired immunity against schistosomes, which afflict >200 million people worldwide. By monitoring bloodstream levels of parasite-gut-derived antigen, we show that from week 10 onwards an established infection with *Schistosoma mansoni* is cleared in an exponential manner, eliciting resistance to reinfection. Secondary challenge at week 42 demonstrates that protection is strong in all animals and complete in some. Antibody profiles suggest that antigens mediating protection are the released products of developing schistosomula. In culture they are killed by addition of rhesus plasma, collected from week 8 post-infection onwards, and even more efficiently with post-challenge plasma. Furthermore, cultured schistosomula lose chromatin activating marks at the transcription start site of genes related to worm development and show decreased expression of genes related to lysosomes and lytic vacuoles involved with autophagy. Overall, our results indicate that enhanced antibody responses against the challenge migrating larvae mediate the naturally acquired protective immunity and will inform the route to an effective vaccine.
A vaccine that provided protection against schistosomiasis for an extended period would be a powerful weapon for control and ultimately eradication of schistosomiasis, a parasitic infection that afflicts over 200 million people worldwide. However, the prolonged residence of adult worms in the host bloodstream, bathed in and feeding on immune effectors, attests to their supremely efficient evasion strategies. Most vaccines attempt to replicate events after the primary exposure to a pathogen that naturally elicits solid immunity but, with chronic infections like schistosomiasis in humans, progress towards the goal has been beset with problems and pitfalls. Initial vaccine experiments involved crude parasite extracts or single immunogenic proteins, generally trialled in the mouse model with limited success. More recently, schistosome transcriptomics, proteomics and genome sequencing changed strategies. Most vaccines attempt to replicate events after the parasite life cycle stages. Finally, we examine the differential antibody levels to four antigen preparations from different parasite life cycle stages. To inform and accelerate the pace of vaccine development, the rhesus macaque model has provided glimpses into the nature of self-cure. To base future work aimed at identifying both the antigens in the worm population. However, the prolonged residence of adult worms in the host bloodstream, bathed in and feeding on immune effectors, attests to their supremely efficient evasion strategies. Most vaccines attempt to replicate events after the primary exposure to a pathogen that naturally elicits solid immunity but, with chronic infections like schistosomiasis in humans, progress towards the goal has been beset with problems and pitfalls. Initial vaccine experiments involved crude parasite extracts or single immunogenic proteins, generally trialled in the mouse model with limited success. More recently, schistosome transcriptomics, proteomics and genome sequencing changed strategies. Most vaccines attempt to replicate events after the parasite life cycle stages. Finally, we examine the differential antibody levels to four antigen preparations from different parasite life cycle stages. To inform and accelerate the pace of vaccine development, the rhesus macaque model has provided glimpses into the nature of self-cure. To base future work aimed at identifying both the antigens in the worm population.
allowing the cure rate to be estimated (Fig. 3b). The demise of the adult worms was a protracted process, taking a mean of 5 weeks for half to be eliminated, and so on in the manner of radioactive decay, out to Wk42. Rh1 with its lower predicted burden had a half-life of 15 weeks, while Rh6 had the most difficulty in dealing with its worm population ($t_{1/2} = 9$ weeks). We stratified the rhesus subjects at the response extremes into Fast and Slow responder groups ($n = 4$ each; Fig. 3a, b), with Rh7 and Rh12 in an intermediate position (Rh9 was preferred to Rh12 in the Fast group because its initial worm burden at Wk10 more closely matched the other three). Regression of the mean values revealed that the $t_{1/2}$ cure rate for the Fast group was 4 weeks versus 7 weeks in the Slow group (Fig. 3c; two-tailed $t$-test for difference of slope, $P < 0.001$).

Data for egg excretion, although less consistent, were subjected to the same analysis, noting that egg excretion had ceased in the majority of animals by Wk24. The declines in egg excretion of individual animals (Fig. 3d, e) did not parallel those of CAA (Fig. 3a). In the Fast group, Rh3 excreted so few eggs that it was omitted from analyses, whilst in the Slow group Rh6 continued egg excretion for the longest period; Rh1 had ceased excretion by Wk24 although its CAA level remained elevated. Regression of the mean Ln EPG values between Wk10 and Wk24 revealed a $t_{1/2}$ of decline in egg excretion for the Fast group of 1.92 weeks versus 3.72 weeks for the Slow group (Fig. 3f; two-tailed $t$-test for difference of slope, $P < 0.05$). A decline in egg excretion occurred at approximately twice the rate for CAA ($t_{1/2}$ ratios CAA/EPG, Fast = 2.1, Slow = 1.85), indicating that the worms spent some weeks in a sterile, non-reproducing state before expiring.

Most worms fail to mature post-challenge. At Wk42, when all macaques were challenged, the majority showed few signs of the infection, and all had regained their initial weights (Fig. 2d). The negligible detection of eggs in the faeces meant events could only be interpreted from CAA values. The low CAA levels at Wk42, adult worms, SWAP).

Probing antibody responses distinguishes egg deposition from worm death. We interrogated the profile of specific IgG production over the 62-week time course of the experiment, using four soluble antigen preparations (Fig. 5). We divided the profiles by life cycle events: skin penetration (Day 0, cercariae, SCAP); migration to, and maturation in the portal system (Wk0–Wk5, schistosomula, SSP); egg deposition (Wk5–Wk10, eggs, SEA); worm death and clearance (Wk10–Wk42, adult worms, SWAP).
Probing the response after primary infection revealed no obvious difference between SCAP and the other three preparations but, all four detected a small increment in antibody level between Wk1 and Wk4, statistically significant for SCAP and SEA (Fig. 5a, c).

The egg deposition phase was marked by a dramatic rise in specific IgG from Wk4 to a peak at Wk8, best exemplified by the SEA preparation (Wk4 cf Wk8; P < 0.001; Fig. 5c), but evident with the other three. Increment in reactivity was greatest for SEA (10.4-fold) and least for SWAP (7.7-fold). There was a positive relationship (Corr. Coeff. 0.79) between the peak reactivity (10.4-fold) and least for SWAP (7.7-fold). There was a positive relationship (Corr. Coeff. 0.79) between the peak reactivity and the predicted adult worm burden, estimated from ref. 43. Rh10, with the highest values, was withdrawn from the study.

The pattern of egg detection in the faeces over the establishment phase for Fast and Slow groups, plus Rh1. Statistical comparisons using one-sided Student t-test with values over Weeks 0–6 not significant; Slow, weeks 6–12, P = 0.00049 (**); Fast, weeks 6–16, P = 0.0029 (***); n = 4 macaques from the Slow and 4 from the Fast groups. The percentage weight loss over the respective periods is indicated by brackets. The level of circulating anodic antigen (CAA) and faecal egg output (eggs per gram, EPG) over the primary time course. Values are means ± SE. Statistical comparisons made using one-sided Student’s t-test are indicated with brackets. For CAA values, P = 0.0009 (Wk6 versus Wk4), P = 0.0082 (Wk12 versus Wk10), P = 0.022 (Wk16 versus Wk12) and P < 0.0001 (Wk24 versus Wk16). For EPG values, P = 0.2023 (Wk8 versus Wk6), P = 0.0219 (Wk10 versus Wk8), P = 0.0109 (Wk10 versus Wk6) and P = 0.024 (Wk16 versus Wk10). Significance P < 0.05 *, <0.01 **, <0.001 ***, <0.0001 ****; n = 11 rhesus macaques, except Rh10. b The level of CAA in the bloodstream as an indicator of worm maturation in each animal over the establishment phase. The colours of plot lines denote stratification into Fast (red), intermediate (black) and Slow (blue) responder groups, plus Rh1 (green) based on cure rate; dotted line (——) indicates the mean of all animals. c The peak value attained by the CAA level in each animal, its week post-infection and the predicted adult worm burden, estimated from ref. 43. Rh10, with the highest values, was withdrawn from the study. d Mean weight change of Fast and Slow groups over the primary time course. The significance of the linear regressions was determined using an F-test with values over Weeks 0–6 not significant; Slow, weeks 6–12, P = 0.00049 (**); Fast, weeks 6–16, P = 0.0029 (***); n = 4 macaques from the Slow and 4 from the Fast groups. The percentage weight loss over the respective periods is indicated by brackets.

Antibody responses after challenge indicate the level of protection induced by self-cure. Antibody reactivity detected by the four preparations after the challenge is plotted as log CAA and EPG, the change observed relative to the Wk42 values, so that anamnestic responses could be evaluated statistically against the primary responses. Inspection of Fig. 5 indicates clearly that the primary
infection profiles were not reprises after the challenge at Wk42. Surprisingly, the SCAP preparation did not detect a recall response to cercarial penetration, with the value at Wk4pc significantly lower than the primary value (Fig. 5e). Similarly, the response detected by SEA at Wk4pc was not different from the primary value (Fig. 5g). In contrast, anti-SWAP responses at Wk1pc, Wk4pc and Wk8pc were significantly greater than after the primary exposure (Fig. 5h). Additionally, the Fast pool reactivity against SSP was three times the primary value, while that of the Slow pool was only 0.5 times (Fig. 5f). The most striking differences were during the egg deposition phase detected by SEA, with the 10.4-fold increment after the primary infection replaced by a 1.4-fold increment, indicating little or no egg deposition (Fig. 5g). At Wk10pc and Wk12pc, the secondary
anti-SWAP response fell sharply to a level below, but not significantly different from, the primary response (Fig. 5h); by Wk16pc it was very significantly lower than after the primary infection ($P < 0.001$). The anti-SSP response detected by the Slow pool peaked at Wk8pc but then declined rapidly out to Wk20pc, while the Fast pool reached baseline by Wk16pc (Fig. 5f).

Elevated granulocyte and lymphocyte populations accompanying self-cure were not observed after the challenge. The haematological changes did not segregate by Fast and Slow responders, so all data were analysed as a single group. Mean haematocrit at Wk0 was 40%, declining to 35.3% by Wk12, a reduction of ~11% ($P < 0.05$; Fig. 6a). It had returned to normal...
Responses detected over the time course of the primary infection (weeks post-infection) with soluble antigen preparations extracted from: a cercariae (soluble cercarial antigen preparation, SCAP); b cultured schistosomula (soluble schistosomula protein, SSP); c eggs (soluble egg antigen, SEA); d adult worms (soluble antigen preparation of adult schistosomes, SWAP). Values for SCAP, SEA and SWAP are means ± SE, n = 11 rhesus macaques, excluding Rh10. Statistical comparisons made using one-sided Student’s t-test are indicated with brackets, and significance $P < 0.05$, $<0.01$, $<0.001$. Those for SSP are responses detected by pools of four Fast (red) and four Slow (blue) responder animals at each time point, without replication due to the low protein yield of the SSP preparation. $n=4$ macaques from the Slow and 4 from the Fast groups.

Fig. 5 Probing antibody responses. Responses detected over the 20 weeks post-challenge using the same four antigen preparations are plotted in: a SCAP; b SSP; c SEA; d SWAP. The antibody reactivity detected is shown as the change observed (Δ), relative to the Week 42 values so that anamnestic responses can be evaluated statistically against the primary responses; data from Weeks 0 to 20 post-infection are replotted as dashed lines for comparison. Values are means ± SE, $n=11$ rhesus macaques (excluding Rh10), except for SSP, as above. Statistical comparisons were made using a one-sided Student’s t-test between the primary and secondary means at the selected time points indicated, with significance $P < 0.05$, $<0.01$, $<0.001$. NS not significant. Source data are provided as a Source Data file.

by Wk24 and overshot to 43.6% at Wk35 ($P < 0.05$). After the challenge, the haematocrit remained level, with a small increase to 43.3% observed at Wk8 ($P < 0.05$). Within the leucocyte populations, the most dramatic change was the marked lymphocytosis, already significant at Wk8 (Fig. 6b; $P < 0.01$), which reached a peak at Wk24 (3.5x the Day 0 value; $P < 0.001$) and remained elevated until the Wk42 challenge when it was still 1.9x higher (Fig. 6b). In marked contrast, challenge parasites elicited only a small lymphocyte response, 1.4x the challenge value at Wk8pc (Fig. 6b, $P = 0.05$, all other values NS). For monocytes, the variation between animals over time made interpretation difficult (Fig. 6b) and no statistically significant changes were observed.

Collectively, granulocytes comprised 78% of circulating leucocytes at Day 0, of which 99% were neutrophils (Fig. 6c). The absolute number of granulocytes increased to a peak at Wk10, with progressively larger proportions of eosinophils (12%) and basophils (4%) versus neutrophils (84%) ($P < 0.01$ for all three over Day 0). Eosinophils peaked (26%) at Wk24 and basophils (4.9%) at Wk12. While eosinophils, and particularly basophils, declined out to Wk42, there was a second spike of neutrophils at Wk41 (significantly higher than Wk24, $P < 0.05$). After the challenge, granulocytes peaked at Wk4pc, comprising 98% neutrophils (Fig. 6c; $P < 0.05$ over the day of the challenge). Eosinophils were never as prominent as after the primary
infection, with wide variation between animals (Wk8pc mean NS versus Wk42/Wk0pc), while basophils did not feature at all.

The inflammatory markers SAA and IP-10 show reciprocal patterns of activity. There was no statistically significant change in either SAA or IP-10 over the first 4 weeks (Fig. 6d). The level of SAA then increased 3.9-fold, from Wk4 to a peak at Wk6, coincident with the onset of egg deposition ($P < 0.01$), followed by a slower decline out to Wk12 ($P < 0.05$). The apparent increase between Wk12 and Wk16 was not significant, but SAA then fell gradually to the background level by Wk32 ($P < 0.01$). After the
challenge, there was no significant upswing in the SAA level after Wk4pc. In contrast, after primary exposure, the level of IP-10 decreased significantly between Wk4 and Wk8 (Fig. 6d; \( P < 0.01 \)), then, as the level of SAA was declining, the level of circulating IP-10 increased 3.9-fold from Wk8 to Wk16 (\( P < 0.001 \)), indicating an approximate 2-week lag in the underlying switching mechanism. Thereafter, IP-10 levels declined out to the initial background level at Wk42 (significant by Wk24; \( P < 0.05 \)). After the secondary challenge, as with SAA, there was no statistically significant change in circulating IP-10 levels.

Plasma from self-curing animals causes enhanced mortality of cultured 3-day-old schistosomula. We assessed the ability of plasma from rhesus macaques over the time course to kill 3-day-old schistosomula, using in vitro coculture assays, with total ATP level relative to untreated control cultures, as the primary readout of larval viability (Fig. 7a). Taken together, the viability of schistosomula incubated with plasma in all cultures did not decline significantly prior to Wk12 (Wk12 < Wk0, \( P < 0.05 \), \( n = 11 \)). However, when Fast and Slow group cultures were analysed separately, a significantly greater killing capacity was evident in the Fast group by Wk8 (\( P < 0.05 \); Fig. 7a). The differential killing continued through Wk10 (\( P < 0.001 \)), Wk12 (\( P < 0.02 \)) and Wk16 (\( P < 0.05 \)) before the capacity of the Slow group plasma caught up, and a plateau was reached at Wk24. After the challenge, there were negligible differences in killing capacity between Fast and Slow group plasma samples, but analysis of the total dataset revealed a further increment in killing over the level at challenge, significant by Wk1pc (Fig. 7b; \( P < 0.01 \)). The value at Wk4pc represents a 50% reduction in schistosomula viability compared to the post-infection levels (from 63 to 31%; \( P < 0.001 \)), and the difference from Wk0pc remained significant from Wk1pc out to Wk20pc (Fig. 7b). As a negative control for the ATP assay, 3-day-old schistosomula were incubated with serum from permisive hamsters, collected at Wk0, Wk12 and Wk22 post-infection with \( S. \) mansoni. These sera had no significant impact on schistosomula viability compared with those grown in medium only (Fig. 7c).

To confirm viability reduction and killing, schistosomula cocultured in vitro with pools of rhesus plasma collected at Wk1 post-infection or Wk10pc from the Fast or Slow groups were stained with PI/FDA and observed under the microscope. Staining with PI and not with FDA confirmed the killing of plasma-treated schistosomula (Supplementary Fig. 5a). Rhesus plasma from Wk10pc killed a higher proportion of schistosomula when compared with Wk1 post-infection (Supplementary Fig. 5b).

Plasma from self-curing animals causes epigenetic and gene expression reprogramming of cultured 3-day-old schistosomula. Application of ChIP-Seq to 3-day-old schistosomula cocultured for 2 days with rhesus plasma collected at Wk10 showed a significant change in H3K4me3 abundance (FDR <0.05) at 76 different genomic regions when compared to Wk0 plasma (Fig. 8a, left panel; affected genes annotated in Supplementary Data 2). GO enrichment analysis (FDR <1%) identified the top ten most enriched Biological Processes (BP) as including actin cytoskeleton maintenance, foregut morphogenesis and negative regulation of autophagy (Fig. 8b left and Supplementary Data 3). An example of two genes (Smp_155780.1 and Smp_207030.1) divergently transcribed from a locus on chromosome 4 is illustrated in Fig. 8c. Both are annotated as ‘GTPase-activating protein-related’, having H3K4me3 marks at their transcription start sites (TSSs) (Fig. 8c, Wk0), which were lost upon exposure of schistosomula to Wk10 plasma (Fig. 8c, Wk10).

When plasma collected at Wk10 was compared with plasma from Wk8 (the earliest time point at which decrease in viability of schistosomula occurred; see Fig. 7a), a change in H3K4me3 abundance (FDR <0.05) at 116 different genomic regions was observed (Fig. 8a, right panel; annotated in Supplementary Data 4). GO enrichment analysis (FDR <1%) identified the top ten most enriched GO Biological Processes as related to genes involved with apoptotic chromosome condensation, epigenetic
regulation of gene expression and chromatin remodelling (Fig. 8b, right and Supplementary Data 5).

To gain further insights into the mechanisms of schistosomula killing, we applied RNA-Seq to 3-day-old schistosomula cocultured in vitro with rhesus plasma collected at Wk0, Wk8 or at Wk1pc and to control schistosomula (Supplementary Data 6). A total of 79 differentially expressed genes (DEGs) were identified when schistosomula were cocultured with Wk8 plasma, compared with control schistosomula but no enriched GO categories were found among them (Supplementary Data 7 and Supplementary Fig. 6). Fifty of these genes (63%) were also differentially expressed in schistosomula cocultured with Wk1pc plasma compared with control schistosomula, together with a further 243 DEGs (Supplementary Data 7 and Supplementary Fig. 6). Remarkably, only three GO categories were enriched among the total of 293 DEGs in the schistosomula cocultured
with Wk1pc plasma, all representing GO cellular components related to late autophagic processes: lytic vacuole, lysosome and vacuole (Supplementary Data 8). Among the ten genes in these three GOs, one (Smp_049150.1, Aspartate aminotransferase) was upregulated. The other nine genes were downregulated (Supplementary Data 7, yellow), including glycosylases, a phospholipase B-like and lysosome-associated membrane proteins. Manual curation identified 19 Smp genes (a total of 26 isoforms) that are orthologs of 15 genes of the early autophagy pathway but the expression level of none of them were affected by the plasma treatments (Supplementary Data 9).

Discussion

Our ability to measure the level of gut-derived CAA regurgitated into the blood as an accurate estimate of parasite burden over the time course provided the key parameter to assess the dynamics of the self-cure process, preferable to faecal egg counts. These were previously considered a poor index of the intensity of infection21, due to the variability of tissue egg accumulation versus egg excretion22, the loss of appetite in some animals21 and diarrhoea in others23. We estimated the inception of blood feeding on Day 8, so allowing a 48 h transition from migrating to erythrocyte-feeding form24, the first schistosomula arrived in the portal system on Day 6. Thereafter, growth was rapid, with increasing expulsion of CAA into the bloodstream. We estimated that the first excretion of eggs occurred at 40 days, comparable to the 36.9 and 39.5 days reported previously21. Allowing for egg development in the tissues (5–6 days)25, the first female worms matured at Day 34, ~26 days after the onset of blood feeding24, so the last arrivals would be maturing around Day 54. Over this period there was minimal observable impact of the schistosomium infection on the animals.

In contrast, from Wk6 onwards, the egg shedding process resulted in weight loss with bleeding into the intestinal lumen and associated dehydration. The 11% reduction in haematocrit by Wk12, agrees with the 14% recorded at 12 weeks after a 750 cercarial exposure21. Similar weight loss (or a lack of weight gain) was reported for S. bovis infections of sheep and goats26, and calves27, along with the elimination of some primary worms, additional retention of eggs in the tissues, and 90% suppression of egg output in surviving worms27. However, unlike rhesus macaques, the immune system in livestock does not deliver the decisive killer blow to S. bovis worms. Why the Fast group should lose more weight over a longer period than the Slow group is unclear since neither CAA level nor faecal egg production suggests a higher worm burden up to Wk10. The logistic analysis indicates that the worm biomass in the Fast group is constrained earlier than in the Slow group. The lower anti-SEA antibody response at Wk8 in the Fast group, indicating a lower fecundity of the females, appears to rule out differential tissue egg retention by Fast group animals (cf.21). Their subsequent history suggests they are the ones best able to control schistosome infection, potentially by mounting a more aggressive immune attack.

The inception of self-cure around Wk10 in almost all animals suggests that antibody levels against key target antigens have reached a threshold ‘immune pressure’18 above which worm survival is affected (see below). The Fast and Slow groups represent the two extremes with ~4-week and ~7-week half-lives. That the intensity of the immune response triggered by the primary infection is the principal host determinant of the elimination process can be inferred from the lower projected adult burden and longest worm half-life (15.5 weeks) of Rh1. Similar anecdotal conclusions about the effect of the initial burden on worm longevity were reached in earlier studies28,29. S. mansoni worms are long-lived in human populations (up to 32 years)30 with a Type I survivorship curve31, whereas the rhesus macaque presents a rare example of a sudden switch to a Type III curve after 10 weeks, with parasite maximum longevity reduced to a few months.

The negative exponential decline in CAA level revealed that the majority of worms in a given animal succumbed rapidly to ‘immune pressure’, whereas a minority showed prolonged survival, pointing to genetic heterogeneity. Previous morphological analysis of surviving worms at 18 weeks showed that while many were no longer feeding on blood and apparently starving to death, others looked normal18. This heterogeneity could result from differential expression of a multiplicity of antigenic targets18 and/or variations in the expression of worm-protective antioxidant proteins32. Our observation of chromatin structure reprogramming in schistosomula exposed to plasma from Wk8 to Wk10, may also reflect a strong immune attack on the schistosomes. Changes in H3K4me3 occupancy at a number of gene loci involved with epigenetic regulation of gene expression and chromatin remodelling suggest that these processes may be early events that lead to worm death. Two of the genes with H3K4me3 chromatin mark, Smp_155780.1 and Smp_207030.1, are more highly expressed in schistosomula and mature adults (>42-day-old)33. They both have a TBC GTPase-activating protein domain, acting on Rab-like GTPases, which play important roles in the regulation of autophagy34, an essential process for animal homoeostasis.

There is morphological evidence for induction of autophagy in schistosomes, in response to stress conditions such as starvation or drug treatment35–37. In addition, some components of the autophagic machinery have recently been described in S. mansoni38, but overall the process is under-researched. During the crucial period of parasite establishment investigated here post-challenge, there are two transitions that involve tissue remodelling. The removal of the spent fecalculi glands during the transformation from cercarial body to skin schistosomulum...
over the first 72 h is coincident with the appearance of acidic compartments representing autophagosomes. Subsequently, in the pulmonary vasculature between 3 and 6 days, the schistosome undergoes a dramatic elongation to facilitate passage through organ capillary beds; this involves a change in circular-longitudinal muscle fibre configuration and removal of the subtegumentary fibrous interstitial layer. Of note, Smp_090090.1, Serpin peptidase inhibitor was the protein-coding gene most highly upregulated (11.4-fold) in Day 6 schistosomula cocultured in vitro with Wk1pc rhesus plasma (Supplementary Data 7), which could impair the activity of the proteases removing the fibrous interstitial layer, so impeding migration. The expression levels of genes encoding early components of the autophagy pathway in Day 6 cultured worms were unaffected by Wk1pc rhesus plasma, whereas the levels of nine out of the ten genes involved in late stages of autophagy were downregulated. A third phase of tissue remodelling (not investigated) provides a further target when the migrating schistosomulum transitions to the blood-feeding juvenile in the portal distributaries of the liver, with the removal of the head capsule muscles and spent head glands. The structural alterations, potentially relying on autophagy, tie in with our observation of plasma-induced autophagy dysfunction in vitro suggesting that immune pressure in vivo may retard larval development and ultimately result in the observed worm starvation, leading to parasite death and to rhesus self-cure.

Regarding the contribution of host cellular responses, studies with other primates have shown very little detectable response of peripheral blood lymphocytes after exposure to a large cercarial dose, either by way of proliferation or cytokine production, until the onset of egg deposition (challenge controls in ref. 41). It is the major antigenic stimulus provided by the egg, which elicits an intense inflammatory response promoting passage through the intestinal tissues to the lumen. Fluctuations in the two circulating markers of inflammation, SAA and IP-10, support a concept of two different forces at play over the primary time course. The absence of changes over the first 4 weeks underscores the minimal inflammatory stimulus provided by the developing parasites, while egg deposition caused a surge in SAA level, depressing IFNγ-induced IP-10 and revealing the emerging dominance of Th2 cytokines. A similar switch was noted in acute human schistosomiasis, related to parasite burden, and is best characterised in the murine model. In rhesus macaques, the Th2 dominance is short-lived with the removal of the antigenic stimulus resulting from the 50% reduction in egg output between Wk10 and Wk12. This takes the brake off IP-10 production, and its rise up to Wk16 signals the death of half the adult worm population. The decline in the level of both markers after Wk16 parallels the resolution of the primary infection.

Circulating granulocytes could also contribute to the demise of adult worms via their consumption of ~21,000 leucocytes/day, potentially attacking worms from within. Indeed, a major function of the >30 proteins secreted from schistosome oesophageal glands is to disable ingested leucocytes and platelets before they reach the gut lumen. Both rhesus macaques self-curing from S. japonicum and in mice exposed to the radiation-attenuated cercarial vaccine, these secreted proteins elicit strong antibody responses that may neutralise their disabling functions, allowing intact leucocytes to reach the gut lumen. All three granulocytes can generate cytotoxic reactive oxygen species and other toxic proteins, and their high numbers at Wk10, coincident with the start of self-cure, suggests a causative link.

Antibody responses to secreted proteins may hold the key to self-cure. The consecutive life cycle stages expose the host to very different amounts of antigen (Summarised in Supplementary Fig. 7a for a 700 cercarial infection). These range from a ~6 µg shot of cercarial secretions through ~12 µg/day from developing blood-feeding worms to ~170 µg/day of eggs exported by females. This >14-fold discrepancy partly explains the dominance of eggs in the stimulation of antibody production. The contribution of eggs was replaced over the Wk10–Wk15 period by ~4000 µg of antigens released by adult worm death. SCAP failed to detect a unique response to primary (or secondary) cercarial exposure, suggesting that its specific constituents play little role in protection. The immune system is next primed by larval secretions detected by the SSP preparation but the muted antibody responses up to Wk4 suggest that these secretions are not strongly immunogenic. In contrast, the dramatic rise in anti-SEA response reflected the dominance of egg-derived proteins, both in quantity and immunogenicity. However, there is little to suggest that these drive the self-cure process since mice vaccinated with live eggs are not protected. Priming by head gland secretions ends with the start of blood feeding, but the contributions of the alimentary tract and tegument continue after Wk5, despite egg dominance, as revealed by the increasing anti-SSP values. Worm death, from Wk10 onwards in all animals, was tracked by the SWAP preparation down to Wk42. Demonstration of the cytotoxic potential of rhesus plasma, revealed by the schistosomula in vitro killing assays, was also evident in Fast responders as early as Wk8, coinciding with the first indication of adult worm stress, but the potency of Slow responder plasma lagged until Wk16. This differential matches the respective cure rates of 4 and 7 weeks between Fast and Slow groups, and points to heterogeneity in the antigens targeted.

We questioned how challenge parasites are eliminated. The very limited response of the self-cured rhesus macaques to a 700 cercarial challenge after Wk42 might at first sight seem surprising. However, it is a testament to the effectiveness of the protection elicited by the self-cure process. Rather than weight loss after challenge, there was a 5% increase, coupled with a small rise in haematocrit at Wk8pc. Both the absence of significant fluctuations in SAA and IP-10 inflammatory markers and the lack of an anti-SEA response after challenge confirm the failure of challenge females to begin oviposition and the absence of perceptible egg shedding into the gut lumen. Furthermore, there was no large biomass of dead juveniles or adults to be disposed of by inflammatory processes, such as those detected after primary exposure.

Antibody responses to the other preparations after the challenge were also muted. The rapid rise in Fast group titre detected by SSP indicates an anamnestic response, peaking at Wk4pc. A minimal commensurate rise in CAA levels suggests that proteins mediating protection are numbered among the specific SSP constituents. Early and stronger secondary response to SWAP, enriched in internal housekeeping proteins, charts the release of somatic antigens ensuing from the death of challenge worms. The increases in killing power of plasma detected as early as Wk1pc, provide crucial evidence for enhanced antibody responses against the migrating larvae. Additionally, peak killing at Wk4pc implicates pre-adults in the portal tract as targets. These conclusions are supported by a hallmark histopathological study, which showed that parasites arriving in the lungs of naïve rhesus macaques provoked minimal inflammatory, whereas in self-cured animals they were initially detained in arterioles and capillaries by small inflammatory foci. The reactions are reminiscent of those described in the lungs of mice exposed to the radiation-attenuated cercarial vaccine versus naïve controls except that in self-cured macaques, a proportion of schistosomula continued their migration to the hepatic portal system but had failed to mature by 6 weeks. In some animals they had all been eliminated by 9 weeks, most likely the equivalents of our Fast responder group. This points to a major distinction between protection in vaccinated mice and self-curing rhesus macaques. Both hosts
generate inflammatory foci that prevent a proportion of larvae from migrating beyond the lungs. Only the rhesus macaque subsequently eliminates blood-feeding stages from the portal tract, potentially via antibody-mediated mechanisms. Understanding the immunological responses and molecular targets underlying the protection elicited by self-cure in the rhesus macaque must surely inform the route to an effective vaccine.

Methods

Ethics statement. Housing conditions of the rhesus macaques and experimental protocols used in the study were in strict accordance with the Ethical Principles in Animal Research established by the Conselho Nacional de Controle de Experimentação Animal (CONCEA) and were approved by the Institutional Animal Care and Use Committee of Instituto Butantan (CEUAIB n° 1388/15). The study was conducted in compliance with the ARRIVE guidelines. The design and execution of the study complied with the recommendations of the Weatherall report (2006). The use of non-human primates in research, in which there are sections dealing with the continued need for primate in schistosomiasis research, particularly in vaccine development. The study also complied with principles set out in the UK NC3Rs Guidelines Primate accommodation, care and use (revised version, October 2017) (http://www.nc3rs.org.uk/primateguidelines). Twelve adult female rhesus macaques (Macaca mulatta) from the captive-breeding colony at the Central Animal Facility at Butantan Institute were group-housed for the whole experiment, permitting social interactions, continuous socialization and colony welfare. The facility is accredited by the Conselho Nacional de Controle de Experimentação Animal (CONCEA). The animals had free access to drinking water and vitamins were provided in a balanced diet, with balanced rations, including various fruits, leafy greens, vegetables, grains (sunflower, corn) and eggs. Environmental enrichment consisted of tyres and plastic barrels, suspended along the top as toys (swings), wooden coops and trunks placed on the floor, as well as fire hoses fixed in the cage ceiling. The rhesus macaques were observed daily to assess welfare using established evaluation criteria, including behaviour (body postures, facial expressions, vocalisations and interactions between animals); food and water intake; stool consistency; clinical signs of pain or discomfort.

Housing conditions of the hamsters and experimental procedures used in this study were also in strict accordance with the Ethical Principles in Animal Research adopted by the CONCEA and the experimental protocol was approved by the Ethics Committee for Animal Experimentation of Butantan Institute (CEUAIB n° 67/48040515). Housing conditions and experimentation with mice followed the recommendations from the Biology Department Ethics Committee, University of York, and experiments were performed on personal (PIL 50/592) and project licences (PPL 60/4340) issued to RAW.

Parasite exposure and sampling regime. The 12 rhesus macaque females used in this study had an age mean of 13.9 ± 2.8 years and a mean weight of 9.3 ± 1.9 kg at the outset. Each animal was exposed to 700 S. mansoni cercariae from a BI isolate (Parasitology Laboratory, Butantan Institute, Brazil) maintained by passage through hamsters (Mesocricetus auratus) and Biomphalaria glabrata snails. Exposure of macaques to cercariae was performed via a metal ring placed on the shaved abdominal skin, for 30 min, under ketamine hydrochloride (10 mg/kg body weight) and midazolam (1 mg/kg body weight) anaesthesia. Of note, accidental leakage of liquid from the metal ring on Rh1 resulted in the animal receiving a lower dose of cercariae. After 30 min exposure, water was pipetted off the skin and inspected for non-penetrant cercariae. A maximum of 5.8% of non-penetrant cercariae was found in Rh4 and there was no significant correlation between blood and serum antibody responses to infection therapy was required for the rhesus macaques as assessed by the plasma level of circulating anodic antigen (CAA) at Wk10 (see below) (Spearman correlation, \( r = 0.1086; P = 0.7366 \)). At Wk42, a 700 cercariae challenge was performed on all macaques, which were then followed up to perfusion at Wk62 (Wk20pc). Blood and faeces were collected at 19 different time points (Fig. 1) from Wk0 (before infection) to Wks 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20, 24, 31, 41, 48, 50, 54, 58 and 62 post-infection, and at Weeks 1 (43), 4 (46), 8 (50), 10 (52), 12 (54), 16 (58) and 20 (62) post-challenge. All the sampling, weighing and infection procedures were performed on anaesthetized animals. Blood was collected from the femoral vein of each animal in: (1) BD Vacutainer CPT tube (BD, 362753), in which plasma was separated and then stored at −80°C. (2) BD Vacutainer EDTA (BD, 367841) for complete blood count. As animals were free-housed, faeces were collected from the rectal ampulla. The eggs are shed from the intestinal tissues into the gut lumen to be voided in the faeces, with concomitant blood and fluid loss. Mild to moderate dehydration was observed in all four Fast groups animals (Rh3, 4, 9 and 11) and one Int group (Rh2). For quantitation of egg excretion therapy was required by three animals (Rh3, 6, 12) showing signs of dehydration at the peak of infection between Wk10 and Wk16. Rh10 became seriously unwell after Wk10 and was withdrawn from the study. Subsequent determination of CAA revealed that it had the highest CAA levels of all animals at Wk10. Five golden hamsters (M. auratus) were infected with 100 cercariae each, to be used as permissive infection controls. Serum from hamsters was collected at Wk0, Wk12 and Wk22, and perfusion was performed at Wk22. Control reference worms were recovered from C57BL/6 mice 7 weeks after exposure to 200 cercariae, fixed in formal saline and processed for microscopy as described in ref. 7.

Estimate of infection intensity. Infection intensity was estimated using two surrogates, eggs per gram of faeces (EPG) and plasma level of circulating anodic antigen (CAA). Prior to infection, eggs were released from 200 cercariae per group in the presence of EPT. EPT was determined using the Percoll technique with 250 mg of faeces as described in ref. 57. For each sample, slides were prepared in triplicates of 150 µL, the eggs counted under a microscope and the mean count multiplied by four to give EPG. Circulating anodic antigen (CAA) was measured applying the up-converting reporter package in R (version 3.6.2)58, graphs were plotted with factoextra (v 1.0.7) and FactoMinR (2.4) packages in R. The entire set of 15 CAA were acquired over 62 weeks. The levels up to Wk10 were analysed by fitting a simple logistic curve to the data using Microsoft Excel (version 15.0.3371.1000) and GraphPad Prism v.8.0 (Supplementary Fig. 1). The levels after Wk10 followed a negative exponential decline and the dataset for Rh10 was fitted by an Ln-transformed, followed by linear regression. This allowed the cure rate to be estimated, where \( t1/2 = In2/ \lambda = 0.693/\text{regression slope} \). The same formula was used to estimate the rate at which egg excretion decreased between Wk10 and Wk24. An estimate of worm burden in adult worm equivalents was back-calculated from data in the literature43 on five adult macaques perfused at Wk44, which had means of 424 worms and 5780 pg/ml CAA, respectively, amounting to 13.6 pg CAA/worm.

Principal components analysis and subject stratification. We knew from previous work with schistosome infections in rhesus macaques that self-cure has a variable aetiology18,19. We, therefore, sought to cluster the ten macaques (excluding Rh1 with lower cercarial exposure and Rh10, withdrawn at Wk10) by performing an unsupervised principal components analysis (PCA) with the prcomp function in R (version 3.6.2)58, graphs were plotted with factoextra (v 1.0.7) and FactoMinR (2.4) packages in R. The entire set of 15 CAA were acquired over 62 weeks. The levels up to Wk10 were analysed by fitting a simple logistic curve to the data using Microsoft Excel (version 15.0.3371.1000) and GraphPad Prism v.8.0 (Supplementary Fig. 1). The levels after Wk10 followed a negative exponential decline and the dataset for Rh10 was fitted by an Ln-transformed, followed by linear regression. This allowed the cure rate to be estimated, where \( t1/2 = In2/ \lambda = 0.693/\text{regression slope} \). The same formula was used to estimate the rate at which egg excretion decreased between Wk10 and Wk24. An estimate of worm burden in adult worm equivalents was back-calculated from data in the literature43 on five adult macaques perfused at Wk44, which had means of 424 worms and 5780 pg/ml CAA, respectively, amounting to 13.6 pg CAA/worm.

Kinetins of immune and antibody responses as well as of haematological changes. We interrogated the profile of specific IgG production over the 62-week time course, using four soluble antigen preparations from the infective cercaria (SCAP), migrating schistosomulum (SSP), mature egg (SEA) and adult worm (SWA). All the preparations share housekeeping proteins of the cytosol and SSA were used as specific controls, but each also Antibody–UCP−LF− assay, as described previously and related to life cycle functions (Summarised in Supplementary Fig. 7b). SSP and SWA were prepared as described in ref. 61. ELISA for SSP and SWAP were performed as described in ref. 60, with minor modifications: microplates were coated overnight at 4°C with a concentration of 0.43 µg/mL (100 µL/well) of the different antigens diluted in carbonate buffer (pH 9.6). For IgG, ELISA plasma was diluted 2000-fold. Care was taken to ensure that the ELISA results were as directly comparable as possible. All ELISAs were performed on the same day, in parallel and using common buffers and substrate reagents. Times for antibody incubations and substrate development were identical for all plates. Levels of IgG against SCAP and SEA were determined by Antibody–UCP−LF− assay, as described previously. In short: plasma samples (1 µL) were diluted in assay buffer and analysed on two LF strips, one containing a Test line of SCAP and the other a Test line of SEA. A
consecutive flow format was used to detect antibody binding to the SCAP or SEA Test line with UCP reporter particles coated with protein-A.

The ability of the animal sera to induce these responses was determined by using two plasma markers, namely serum amyloid A (SAA) and interferon-y inducible protein 10 (IP-10). SAA is an acute-phase protein of the short pentraxin family, produced in hepatocytes, primarily in response to IL-6 (ref. 68). IP-10 (C-X-C motif) is a chemokine produced by several leukocyte types in response to IFNγ stimulation, which in turn binds to the CXCR3 receptor, recruiting activated Th1 lymphocytes to sites of inflammation9. The levels of SAA and IP-10 were measured using the UCP-LF platform as previously described89, with two different test strips specific for SAA and IP-10. The antibodies used were: mouse monoclonal for SAA sandwich: on the LF strip mAb anti-SAA1 clone SAA15 (NB100-73071); the LF conjugate with mAb clone SAA1 [NB100-73071] (both from Novus Biologicals); and mouse monoclonal for IP-10 sandwich: on the LF strip mAb anti-IP-10 clone C-55 [879.950], the UF conjugate with mAb clone B-C50 [855.420] (both from Diacron Research).

Erythrocyte and leucocyte counting, as well as haemoglobin determination, were performed automatically in an ABX Pentra XL 80 instrument (Horiba). Differential leucocyte counts were performed on one hundred cells in a blood smear using an Eclipse E200 microscope (Nikon). Haematocrit was determined with the micro-capillary technique, using a Famen 3400 centrifuge.

Recovery and morphological analysis of surviving worms. Perfusion were performed at Wk62 (Wk20pc). Rhesus macaques were initially sedated with a mixture of ketamine hydrochloride and xylazine for blood and faeces collection, as described above. Heparin (10,000 U) was then injected via the femoral vein and after 5 min the animals were euthanized with pentobarbital (21 mg/kg) and ketamine hydrochloride (36 mg/kg) as previously described90,91. RPMI-1640 medium (Gibco, Life Technologies) buffered with 10 mM HEPES was infused into the aorta via a percutaneous puncture, and the perfusate was collected at the portal vein outlet. The recovered parasites were harvested from the perfusate, washed in PBS, counted per rhesus and fixed in Karynny’s solution (16% paraformaldehyde, 0.2 M sodium phosphate buffer, 50% glutaraldehyde, 0.2 M cacodylate buffer, pH 7.4) for confocal microscopy. Worms were prepared for confocal microscopy by staining in Langeron’s Carmin, dehydration and mounting as previously described87,88. Optical slices were obtained on a Zeiss LSM710 confocal microscope, with excitation at 514 nm from a 25-mW argon-ion laser and a 585 nm long-pass emission filter.

Schistosoma in vitro culture. Cercariae shed from infected snails were cooled on ice for 30 min and collected by centrifugation. Schistosomula were obtained by mechanical transformation of cercariae, and separation of their bodies as previously described72. The newly transformed schistosomula (NTS) were maintained for 72 h, before coculture, in M169 medium (Vitrocell, cat number 00464) supplemented with penicillin/streptomycin, amphotericin, gentamicin (Vitrocell, cat number 00146), 1 µM serotonin, 0.5 µM hypoxanthine, 1 µM hydrocortisone and 25 mM sodium phosphate buffer, pH 7.4 (M169). The newly transformed schistosomula (NTS) were maintained in vitro culture conditions, as described above. Heparin (10,000 IU) was then injected via the femoral vein and after 5 min the animals were euthanized with pentobarbital (21 mg/kg) and ketamine hydrochloride (36 mg/kg) as previously described89,92. RPMI-1640 medium (Gibco, Life Technologies) buffered with 10 mM HEPES was infused into the aorta via a percutaneous puncture, and the perfusate was collected at the portal vein outlet. The recovered parasites were harvested from the perfusate, washed in PBS, counted per rhesus and fixed in Karynny’s solution (16% paraformaldehyde, 0.2 M sodium phosphate buffer, 50% glutaraldehyde, 0.2 M cacodylate buffer, pH 7.4) for confocal microscopy. Worms were prepared for confocal microscopy by staining in Langeron’s Carmin, dehydration and mounting as previously described87,88. Optical slices were obtained on a Zeiss LSM710 confocal microscope, with excitation at 514 nm from a 25-mW argon-ion laser and a 585 nm long-pass emission filter.
were performed as described for Chip-Seq. RNA-Seq reads were mapped using STAR89 (v 2.7.3a) against the S. mansoni genome PRJEA36577 (v7) retrieved from WormBase (schistosoma_mansoni.PRJEA36577.WBPS14.genomic_softmasked.fa) and the overall average mapping rate was 98% (Supplementary Data 12). Read counting was performed with RSEM89 (v 1.3.1) using a previously published transcriptome annotation84. Read count values (Supplementary Data 6) are shown as represented GOs (FDR < 5%).

**References**

1. Mutapi, F., Maizels, R., Fenwick, A. & Woolhouse, M. Human schistosomiasis in the post mass drug administration era. Lancet Infect. Dis. 17, e42–e48 (2017).
2. Wilson, R. A. & Coulson, P. S. Immune effector mechanisms against schistosomiasis: looking for a chink in the parasite’s armour. Trends Parasitol. 25, 423–431 (2009).
3. Wilson, R. A., Li, X. H. & Castro-Borges, W. Schistosome vaccines: problems, pitfalls and prospects. Emerg. Top. Life Sci. 1, 641–650 (2017).
4. Verjovski-Almeida, S. et al. Transcriptome analysis of the acoelomate human D. discombaculum. J. Helminthol. 74, 237–248 (2003).
5. Curwen, R. S., Ashton, P. D., Johnston, D. A. & Wilson, R. A. The Schistosoma mansoni soluble proteome: a comparison across four life-cycle stages. Mol. Biochem. Parasitol. 138, 57–66 (2004).
6. Berriman, M. et al. The genome of the blood fluke Schistosoma japonicum. Nature 460, 352–358 (2009).
7. Wilson, R. A. Proteomics at the schistosome-mammalian host interface: any prospects for diagnostics or vaccines? Parasitology 139, 1178–1194 (2012).
8. Rondin, C. et al. Safety and efficacy of the r5826GST urinary schistosomiasis vaccine: a phase 3 randomized, controlled trial in Senegalese children. PLoS Negl. Trop. Dis. 12, e0006968 (2018).
9. Hotez, P. J., Bottazzi, M. E., Bethony, J. & Diemert, D. D. Advancing the development of a human schistosomiasis vaccine. Trends Parasitol. 35, 104–108 (2019).
10. Siddiqui, A. A. & Siddiqui, Z. S. Sm-p80-based schistosomiasis vaccine: preparation for human clinical trials. Trends Parasitol. 33, 194–201 (2017).
11. Crossner, C. et al. Systematic screening of 96 Schistosoma mansoni cell-surface and secreted antigens does not identify any strongly protective vaccine candidate in a mouse model of infection. Wellcome Open Res. 4, 159 (2019).
12. Wilson, R. A. & Coulson, P. S. in Parasitic Flatworms: Molecular Biology, Biochemistry, Immunology and Physiology (eds Maule, A. G. & Marks, N.J.) Ch. 16 (CABI, 2006).
13. Melenev, H. E. & Moore, D. V. Observations on immunity to superinfection with Schistosoma mansoni and S. haematobium in monkeys. Exp. Parasitol. 3, 128–139 (1954).
14. Vogel, H. Acquired resistance to Schistosoma infection in experimental animals. Bull. World Health Organ. 18, 1097–1103 (1958).
43. Fallon, P. G. et al. Juvenile rhesus monkeys have lower type 2 cytokine responses than adults after primary infection with Schistosoma mansoni. J. Infect. Dis. 187, 935–945 (2003).

44. Caldas, I. R. et al. Human schistosomiasis mansoni: immune responses during acute and chronic phases of the infection. Acta Trop. 108, 109–117 (2008).

45. Pearce, E. J., Caspar, P., Grzych, J. M., Lewis, F. A. & Sher, A. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth. J. Exp. Med. 173, 139–166 (1991).

46. Lawrence, J. D. The ingestion of red blood cells by Schistosoma mansoni. J. Parasitol. 59, 60–63 (1973).

47. Li, X. H. et al. The schistosome oesophageal gland: initiator of blood processing. PLoS Negl. Trop. Dis. 9, e0002472 (2015).

48. Wilson, R. A. et al. The schistosome esophagus is a ‘hotspot’ for microoxen and cytoskeletal hallmark gene expression: implications for blood processing. PLoS Negl. Trop. Dis. 9, e0002476 (2015).

49. Li, X. H. et al. Mapping the epithetes of Schistosoma japonicum esophageal gland proteins for incorporation into vaccine constructs. PLoS ONE 15, e0229542 (2020).

50. Farias, L. P. et al. Epitope mapping of exposed tegument and alimentary tract proteomes of Schistosoma mansoni and Schistosoma japonicum. PLoS Negl. Trop. Dis. 11, e0006352 (2017).

51. Wangwiwatsin, A. et al. Transcriptome of the parasitic Schistosoma mansoni during intra-mammalian development. PLoS Negl. Trop. Dis. 14, e0007743 (2020).

52. Stavrici, T. M., Bjerke, L. O., Wilson, R. A. & Coulson, P. S. Antibodies elicited by the secretions from schistosome cercariae and eggs are predominantly against glycian epitopes. Parasite Immunol. 30, 554–562 (2008).

53. Harrison, R. A., Bickle, Q. & Doenhoff, M. J. Factors affecting the acquisition of resistance against Schistosoma mansoni in the mouse. Evidence that the mechanisms which mediate resistance during early patent infections may lack linear correlation. Parasitol. Immunol. 8, 110–116 (1988).

54. Von Lichtenberg, F. & Ritchie, L. S. Cellular resistance against schistosomula of Schistosoma mansoni in Macaca mulatta monkeys following prolonged infections. Am. J. Trop. Med. Hyg. 10, 859–869 (1961).

55. Crabtree, J. E. & Wilson, R. A. The role of pulmonary cellular reactions in the resistance of vaccinated mice to Schistosoma mansoni. Parasite Immunol. 8, 265–285 (1986).

56. Smythies, L. E., Betts, C., Coulson, P. S., Dowling, M. A. & Wilson, R. A. Kinetics and mechanism of effector focus formation in the lungs of mice vaccinated with irradiated cercariae of Schistosoma mansoni. Parasite Immunol. 18, 359–369 (1996).

57. Eberl, M. et al. A novel and sensitive method to monitor helminth infections by fecal sampling. Acta Trop. 83, 183–187 (2002).

58. Corstjens, P. L. et al. Multi-center evaluation of a user-friendly lateral strip assays as user-friendly tools for monitoring helminth infections. Acta Trop. 179, 139–147 (2018).

59. van Hooij, A. et al. Quantitative lateral flow strip assays as user-friendly tools for monitoring helminth infections. Acta Trop. 187, 12–18 (2019).

60. Amur, S., LaVange, L., Zineh, I., Buckman-Garner, S. & Woodcock, J. Rapid assay format for multiplex detection of humoral immunological signatures of active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB. J. Infect. Dis. 198, 1559–1567 (2008).

61. Basch, P. et al. Cultivation of Schistosoma mansoni in vitro. I. Establishment of cultures from cercariae and development until pairing. J. Parasitol. 67, 179–185 (1981).

62. Amaral, M. et al. Long non-coding RNA levels can be modulated by 5-azacytidine in Schistosoma mansoni. Sci. Rep. 10, 21565 (2020).

63. peak, E., Chalmins, I. W. & Hoffmann, K. F. Development and validation of a quantitative, high-throughput, fluorescent-based bioassay to detect schistosomiasis viability. PLoS Negl. Trop. Dis. 4, e759 (2010).

64. Roquis, D. et al. Histone methylation changes are required for life cycle progression in the human parasite Schistosoma mansoni. PLoS Pathog. 14, e1007068 (2018).

65. Chen, S., Zhou, Y., Chen, Y. & Gu, J. Fastp: an ultra-fast all-in-one FASTQ quality processor. Bioinformatics 34, 8884–8891 (2018).

66. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359 (2012).

67. Li, H. et al. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079 (2009).

68. Suzuki, H., Osawa, T., Fujioka, Y. & Noda, N. N. Structural biology of the core autophagy machinery. Curr. Opin. Struct. Biol. 34, 93–110 (2018).

69. Maciel, L. F. et al. Weighted gene co-expression analyses point to long non-coding RNA Hub genes at different Schistosoma mansoni life-cycle stages. Front. Genet. 10, 823 (2019).

70. Ross-Innes, C. S. et al. Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. Nature 481, 389–393 (2012).

71. Huerta-Cepas, J. et al. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res. 47, D309–D314 (2019).

72. Maere, S., Heymans, K. & Kuiper, M. BiNGO: a cytoscape plugin to overlay representation of gene ontology categories in biological networks. Bioinformatics 21, 3448–3449 (2005).

73. Supek, F., Bosnjak, M., Skunca, N. & Smuc, T. REVIGO summarizes and visualizes long lists of gene ontology terms. PLoS ONE 6, e21800 (2011).

74. Dobin, A. et al. STAR: ultrafast and universal RNA-seq aligner. Bioinformatics 29, 15–21 (2013).

75. Li, B. & Dewey, C. N. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinform. 12, 323 (2011).

76. Suzuki, H., Osawa, T., Fujioka, Y. & Noda, N. N. Structural biology of the core autophagy machinery. Curr. Opin. Struct. Biol. 34, 93–110 (2018).

77. Liu, R. et al. Why weight? Modeling sample and observational level variability improves power in RNA-seq analyses. Nucleic Acids Res. 43, e95 (2015).

78. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140 (2010).

79. Leek, J. T. ss nugrep: removing batch effects and other unwanted noise from sequencing data. Nucleic Acids Res. 42, e161 (2014).

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