Controlled human infection models as a tool for malaria and schistosomiasis vaccine research

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CHAPTER 7

A controlled human *Schistosoma mansoni* infection model to advance novel drugs, vaccines and diagnostics

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Abstract

Schistosomiasis treatment relies on the use of a single drug, praziquantel, which is insufficient to control transmission in highly endemic areas[1]. Novel medicines and vaccines are urgently needed[2,3]. An experimental human model for schistosomiasis could accelerate the development of these products. We performed a dose-escalating clinical safety trial in 17 volunteers with male *Schistosoma mansoni* cercariae, which do not produce eggs (clinicaltrials.gov NCT02755324), at the Leiden University Medical Center, the Netherlands. The primary endpoints were adverse events and infectivity. We found a dose-related increase in adverse events related to acute schistosomiasis syndrome, which occurred in 9 of 17 volunteers. Overall, 5 volunteers (all 3 of the high dose group and 2 of 11 of the medium dose group) reported severe adverse events. Worm-derived circulating anodic antigen, the biomarker of the primary infection endpoint, peaked in 82% of volunteers at 3–10 weeks following exposure. All volunteers showed IgM and IgG1 seroconversion and worm-specific cytokine production by $CD4^+$ T cells. All volunteers were cured with praziquantel provided at 12 weeks after exposure. Infection with 20 *Schistosoma mansoni* cercariae led to severe adverse events in 18% of volunteers and high infection rates. This infection model paves the way for fast-track product development for treatment and prevention of schistosomiasis.

Introduction

Worldwide, 290 million people are infected with schistosomes, mainly *Schistosoma haematobium* and *Schistosoma mansoni*[4]. The endemicity is determined by the presence of the fresh water snail intermediate host. Snail-derived cercariae penetrate the human skin and migrate into the vascular system, where mature male and female worms mate and produce ~300 eggs per day. *S. mansoni* eggs provoke inflammatory responses, which can lead to liver cirrhosis and portal hypertension[5].

Current treatment and control of schistosomiasis relies on the use of a single drug, praziquantel. Mass drug administration with praziquantel does not protect from reinfection[1] and thus provides insufficient control in highly endemic areas, creating the need for a vaccine[2].

Several schistosome antigens have been put forward as possible *S. mansoni* vaccine candidates, of which three are in clinical development: Sm-TSP-2, rSm14/GLA-SE and Sm-p80[6–8]. These candidates aim for >40% reduction in worm load for World Health Organization endorsement[9], but higher levels are preferred[10,11]. To obtain efficacy data, large phase II and III field trials in *Schistosoma*-endemic areas are needed[12]. In addition, increasing concerns of praziquantel resistance create a need for anti-schistosomal drug development[3]. Controlled human infection (CHI) trials can select drug and vaccine candidates early in clinical development and help prevent late clinical failure[12]. We thus aimed to develop a schistosome CHI model to aid vaccine and drug development and better characterize human anti-schistosome immune responses.

Between September 2016 and January 2018, 35 healthy adult volunteers were screened, of which 17 were included in the trial and completed follow up (Fig. 1). Baseline demographics between dose groups were comparable (Supplementary Tables 1 and 2).

Volunteers were exposed to 10, 20 or 30 cercariae in water on the forearm skin for 30 min, after which they were followed bi-weekly for adverse events and infectivity. After exposure, water was pipetted off the skin and inspected for remaining cercariae. We found tails of roughly half the number of the cercariae that they were exposed to, with clear differences between exposure groups (r = 0.70, P = 0.002; Supplementary Table 1).

Results

Safety

The majority of volunteers (15 of 17, 88%) experienced pruritus during or after exposure, but no topical treatment was required (Supplementary Table 3). All but one volunteer (16 of 17, 94%) developed a mild local cercarial dermatitis within 2 d after exposure (Fig. 2a–c), which lasted longer in higher dose groups (10 cercariae: median 1 week (range 1–2); 20 cercariae: 3 weeks (1–9); 30 cercariae: 6 weeks (2–7), r = 0.45, P = 0.07).

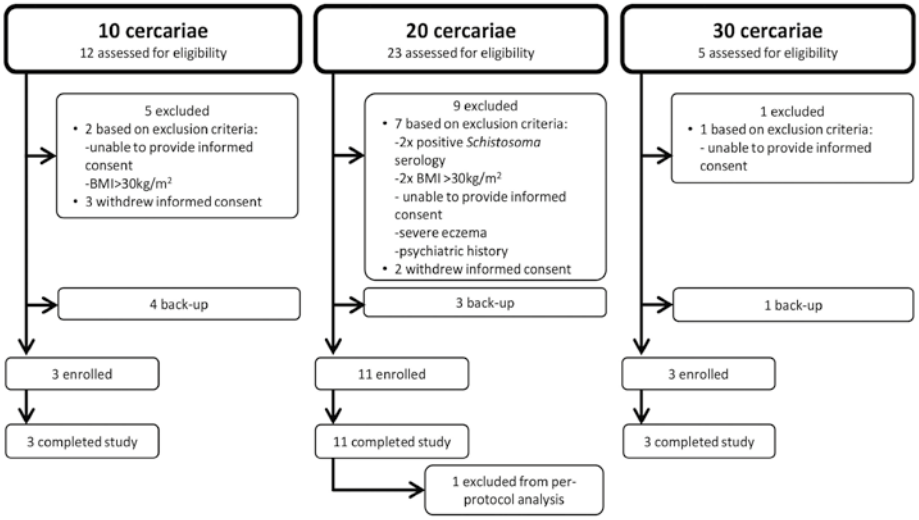

Figure 1. Study flow diagram. The inclusion and exclusion of volunteers. The first group (*n* = 3 volunteers) was exposed to 10 cercariae and the second group (n = 3 volunteers) to 30 cercariae. Thereafter the dose was de-escalated to 20 cercariae for the third group (n = 3 volunteers) and validated in a fourth group (*n* = 8 volunteers). In total eight volunteers served as backup for included volunteers in case an included volunteer might drop out before exposure to cercariae.

There were no serious adverse events (AEs) (an event that is life-threatening or requires hospitalization), but nine severe related AEs (resulting in the inability to perform daily activity) were reported in volunteers from the 20 (*n* = 2) or 30 cercariae (*n* = 3) group. Seven of these severe AEs were symptoms of an acute schistosomiasis syndrome (*n* = 5). In the 30 cercariae group all volunteers (*n* = 3) experienced severe AEs, starting 2.5 to 5.0 weeks after exposure (Fig. 2d–f) as follows: headache (*n* = 2), fever (*n* = 2), syncope (*n* = 1), nausea (*n* = 1) and elevated liver enzymes (*n* = 1) (Supplementary Table 3). In one volunteer this episode was followed by mild to moderate headaches, malaise, fatigue and nocturnal sweats for up to 6 weeks as previously described[13], but steroid treatment was declined. Given the burden of these prolonged symptoms of an acute schistosomiasis syndrome (Katayama symptoms), defined as moderate to severe symptoms of malaise, fatigue, fever, night sweats, flu-like symptoms or headache, between 2 to 7 weeks after exposure, the dose was de-escalated to 20 cercariae. Subsequently, 2 of 11 (18%) volunteers reported one severe symptom of an acute schistosomiasis syndrome (headache, nocturnal fever and sweats).

In addition to these five volunteers with severe AEs there were four volunteers, all exposed to 20 cercariae, with moderate symptoms of an acute schistosomiasis syndrome (flu-like symptoms, *n* = 3; and malaise, *n* = 2). Eight volunteers did not experience any symptoms of an acute schistosomiasis syndrome.

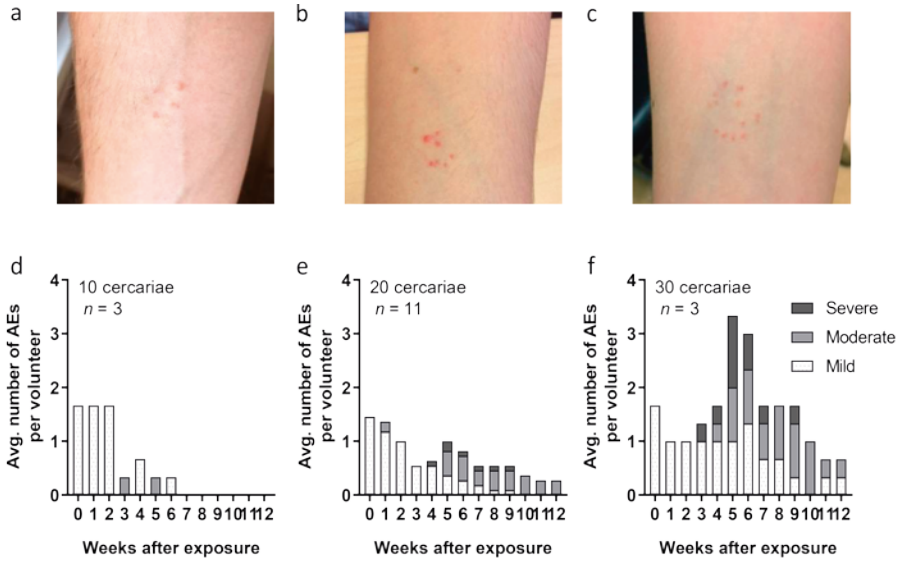

Figure 2. Adverse events. a–c, Pictures of volunteers with the most pronounced cercarial dermatitis taken 5 d after infection from the group infected with 10 cercariae (**a**), 20 cercariae (**b**) or 30 cercariae (**c**). **d–f**, The average number of related AEs per volunteer at each week for volunteers exposed to 10 cercariae (**d**), 20 cercariae (**e**) or 30 cercariae (**f**). For each AE the highest grading score is plotted. White bar, mild AE; light gray bar, moderate AE; dark gray bar, severe AE.

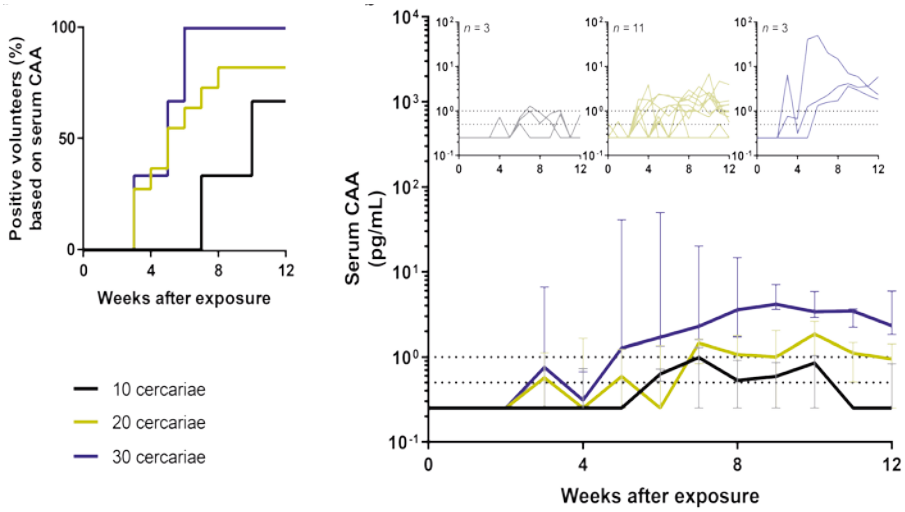

Figure 3. Pre-patent period and serum CAA levels. a, The time to patency (serum CAA level > 1 pg ml^{-1}) in weeks per dose group (log-rank test, $P = 0.21$). **b**, The serum CAA levels for individual volunteers in pg ml^{-1} (top) and the median serum CAA levels and interquartile range for groups infected with 10, 20 or 30 cercariae (bottom). The gray, yellow and blue lines represent data of individual volunteers or groups exposed to 10 ($n = 3$), 20 ($n = 11$) or 30 ($n = 3$) cercariae respectively.

Eosinophils

Eosinophils increased in 11 of 17 (65%) volunteers peaking between week 2 and 8 after infection (0.1–8.4 × 10^9 l^{-1}; Extended Data Fig. 1). Eosinophils were not related to the dose or symptoms (Extended Data Fig. 2a).

Infection rates by antigen detection assays

Both worm-excreted circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) were measured to determine the presence and degree of infection. In two of three volunteers exposed to 10 cercariae the serum CAA was higher than 1 pg ml^{-1}. In the higher dose groups, 9 out of 11 (82%) volunteers exposed to 20 cercariae and all volunteers exposed to 30 cercariae crossed this threshold. The time to patency was comparable between these groups (range 3–8 weeks, Fig. 3a).

Cercarial dose and serum CAA levels were related (10 cercariae median at week 7–12: 0.4 pg ml^{-1} (range 0.3–0.8); 20 cercariae: 1.2 pg ml^{-1} (0.3–1.9); and 30 cercariae: 3.6 pg ml^{-1} (2.0–6.5), $r = 0.70$, $P = 0.002$) (Fig. 3b). The volunteer experiencing the most severe AEs had at least sevenfold higher serum CAA levels (maximum 49.9 pg ml^{-1}) than other volunteers. There was no significant correlation between serum CAA levels and symptoms of an acute schistosomiasis syndrome (Extended Data Fig. 2b) or eosinophils (data not shown).

Urine CAA levels were variable (Extended Data Fig. 3a,b), but correlated with serum CAA ($r = 0.58$, $P < 0.0001$). The point-of-care rapid test for CCA (POC-CCA) was positive in 12% (2 of 17) of volunteers within 12 weeks after exposure and did not correlate with serum or urine CAA levels.

Praziquantel treatment

After a single 40 mg kg^{-1} dose of praziquantel treatment 12 weeks after exposure, serum CAA levels dropped below the detection limit in 8 out of 14 (57%) positive volunteers. The remaining 43% of volunteers were treated again with praziquantel, after which all remained undetectable until 1 year after exposure (Extended Data Fig. 4a–c).

Serology

All volunteers showed seroconversion of IgM against adult worms by immunofluorescence assay (IFA) (Fig. 4a,b). Seroconversion became apparent at week 4 in two volunteers and by week 6 in all. In addition, seven volunteers showed anti-soluble egg antigen (SEA) IgG seroconversion respectively at week 4 (1 of 16), week 12 (1 of 16) or week 20 (5 of 16). The absence of eggs was confirmed by a negative feces *Schistosoma* PCR at week 12 in all volunteers.

All volunteers showed an increase in adult worm antigen (AWA)-specific total IgG over time, with seroconversion above 2× s.d. of all baseline values in 12 of 16 volunteers at week 20. These responses were primarily IgG1, with seroconversion in all volunteers by week 16 (Fig. 4c,d).

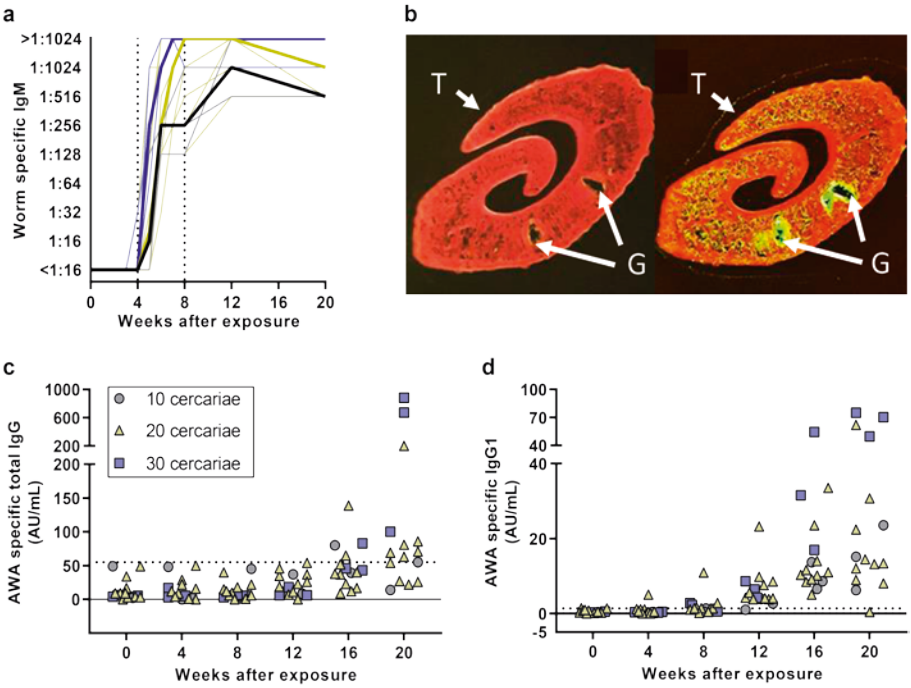

Figure 4. Humoral immune response. a, IgM against adult worm antigens over time. The gray, yellow and blue lines represent data of individual volunteers exposed to 10 (*n* = 3), 20 (*n* = 10) or 30 (*n* = 3) cercariae respectively and the darker lines represent the median of each group. **b**, IFA showing reactivity of volunteer IgM antibodies to Rossman-fixed *S. mansoni* adult worm sections (right) and pre-exposure negative serum (left). T, tegument; G, gastrodermis. **c**, AWA-specific total IgG for individual volunteers over time. **d**, AWA-specific IgG1 for individual volunteers over time. The gray circles, yellow triangles and blue squares represent data of individual volunteers exposed to 10 (*n* = 3), 20 (*n* = 10) or 30 (*n* = 3) cercariae respectively.

There was a clear dose response in AWA-specific total IgG and IgG1 levels (IgG week 20: $r = 0.70$, $P = 0.003$; IgG1 week 16: $r = 0.56$, $P = 0.02$), and a trend toward higher IgG1 levels and symptoms of an acute schistosomiasis syndrome ($P = 0.08$) (Extended Data Fig. 2c). No changes over time were found in total IgE or AWA-specific IgE and IgG4 compared to baseline (data not shown).

Cytokines

Cytokine and chemokine measurements in serum revealed increases in innate chemokines interferon (IFN)-γ- inducible protein (IP)-10 (Fig. 5a,b) and macrophage inflammatory protein (MIP)-1β (Fig. 5c), the latter of which was significantly higher in volunteers with symptoms of an acute schistosomiasis syndrome ($P = 0.01$ at week 8, Fig. 5d). There were no detectable changes in the other circulating chemokines or cytokines measured in serum (data not shown).

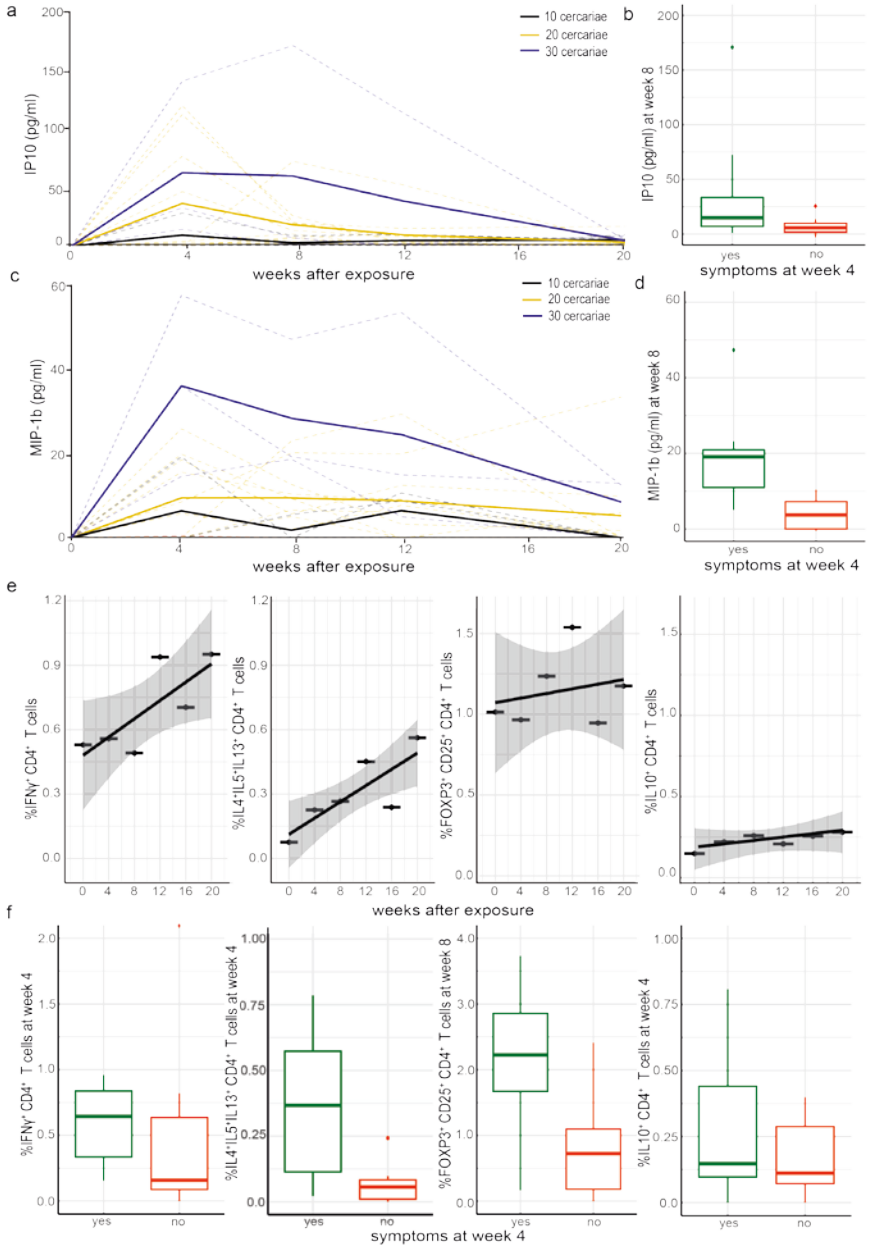

Figure 5. Cytokine responses. a, Ex vivo IP-10 over time for individual volunteers (dotted line) and the mean of each group (line). **b**, IP-10 levels at week 8 in volunteers with (yes, *n* = 8) or without (no, *n* = 8) symptoms of an acute schistosomiasis syndrome (two-sided Mann–Whitney *U*-test, *P* = 0.16). **c**, Ex vivo MIP-1β over time for individual volunteers (dotted line) and the mean of each group (line). **d**, MIP-1β levels at week 8 in volunteers with (yes, *n* = 8) or without (no, *n* = 8) symptoms of an acute schistosomiasis syndrome at week 4 (two-sided Mann–Whitney *U*-test, *P* = 0.01). **e**, The percentage of IFN-γ-producing CD4⁺ T cells (*P* = 0.01), Th2 cytokine (IL-4, IL-5 and IL-13)-producing CD4⁺ T cells

(P = 0.004), FOXP3$^+$CD25$^+$CD4$^+$ T cells (P = 0.49) and IL-10-producing CD4$^+$ T cells (P = 0.91) over time for all volunteers (n = 16), showing average of all volunteers at each week and fitted linear regression line with 95% confidence interval (linear mixed model with two-sided Student's t-test, using the Satterthwaite's degrees of freedom method). **f**, The percentage of IFN-γ-producing CD4$^+$ T cells (P = 0.28), Th2 cytokine (IL-4, IL-5 and IL-13)-producing CD4+ T cells (P = 0.01), FOXP3$^+$CD25$^+$CD4$^+$T cells (P = 0.02) and IL-10-producing CD4$^+$ T cells (0.56) in volunteers with (yes, n = 8) or without (no, n = 8) symptoms of an acute schistosomiasis syndrome. In all boxplots, the lower and higher hinge are respectively the first and third quartiles and the middle line is the median (second quartile). The whiskers extend from the hinge to the largest value no further than 1.5× the interquartile range or distance between the first and third quartile. The largest and lowest visible points beyond the whiskers extend this range and can then be considered, respectively, the maxima and minima.

Cellular immunology

Overall, the frequency of antigen-specific IFN-γ (P = 0.01) and Th2 cytokine (interleukin (IL)-4, IL-5 and IL-13, P = 0.004)-producing CD4$^+$ T cells increased over time (Fig. 5e and Extended Data Fig. 5a,b), but FOXP3$^+$CD25$^+$CD4$^+$ regulatory T cells (P = 0.49) or the production of the regulatory cytokine IL-10 (P = 0.91, Fig. 5e) did not. However, in volunteers with symptoms of an acute schistosomiasis syndrome, both IFN-γ and Th2 cytokine-producing CD4$^+$ T cells and CD25$^+$FOXP3$^+$ regulatory T cells were higher at week 4 and week 8 respectively, although not significantly for all (P = 0.28, P = 0.01 and P = 0.02, respectively; Fig. 5f). There were no differences in IL-10-cytokine-producing CD4$^+$ T cells at week 4 (Fig. 5f). At all timepoints 17% of IFN- γ$^+$CD4$^+$ T cells also produced IL-2.

Data integration

To understand which immunological and microbiological datasets were associated with the occurrence of symptoms of an acute schistosomiasis syndrome, we performed data integration using parallel generalized canonical correlation and partial least squares discriminant analysis. Eosinophil data decreased model accuracy and were thus removed (Extended Data Fig. 6a,b). At baseline, 9 of 16 (56.3%) participants were accurately predicted by the model with leave-one-out cross-validation, which increased to 13 of 16 (81.3%) correct classifications at week 12 and further to 15 of 16 (93.8%) when data from all timepoints were included (Fig. 6a,b). Thus, the model was able to accurately classify participants by the presence of an acute schistosomiasis syndrome. Permutation analysis confirmed that symptoms were strongly associated with the measured immunological and microbiological parameters over the infection course (n = 1,000; 99.6th percentile, Extended Data Fig. 6c). We identified Th2 cytokines at week 4 and FOXP3 regulatory T cells at weeks 12 and 16, MIP-1β at weeks 8 and 12 and levels of serum CAA at week 9 as important features elevated in symptomatic participants by leave-one-out cross-validation (Fig. 6c). Levels of Th2 cytokines upon stimulation at week 4 correlated with serum CAA at week 9, whereas concentrations of MIP-1β in plasma correlated with FOXP3 regulatory T cell numbers (Extended Data Fig. 6d).

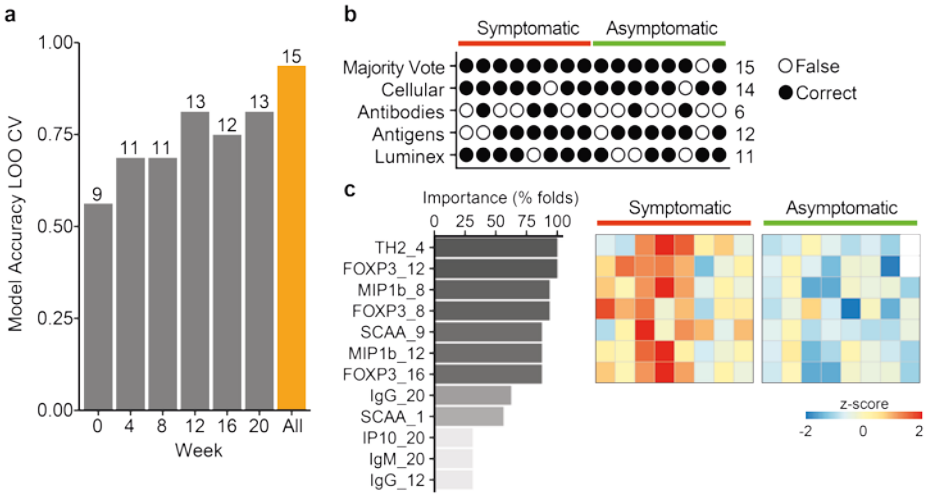

Figure 6. Immunological and microbiological data integration. Cellular, antibody, antigen and Luminex data were combined with generalized canonical correlation analysis and simultaneously associated with presence of symptoms through discriminant analysis. **a**, Proportion of correct predictions of each model assessed using leave-one-out cross-validation. Gray bars indicate data from one specific timepoint and orange bars indicate the model, including all data across timepoints. The number of correct predictions is indicated above each bar. **b**, Individual predictions across folds for the full model per dataset, including majority vote, with weighing to break ties. Each symbol represents one prediction, with volunteers in columns and datasets in rows. Filled circles indicate a correct prediction and open circles a false prediction. Volunteers with (red, *n* = 8) or without (green, *n* = 8) symptoms of acute schistosomiasis syndrome are indicated, as are the number of correct predictions per dataset. **c**, Consensus features selected in at least 25% of the folds are indicated and ranked by frequency of presence among folds. The number after each feature indicates the week of measurement. The *z* score-normalized levels of the seven features present in at least 75% of the folds are indicated in the heat map, where each column corresponds to one volunteer. Missing data points from one asymptomatic participant are depicted in white.

Discussion

This pilot study shows that experimental exposure to 20 male cercariae results in a detectable and well-tolerated *S. mansoni* infection in 82% of volunteers. This infection rate resembles that of other human infection models[14]. All volunteers were cured after 1–2 doses of praziquantel. The dose-escalating design of the study revealed a concomitant increase in AEs. The occurrence of a severe acute schistosomiasis (Katayama) syndrome in one volunteer prompted us to lower the dose. At a dose of 20 cercariae, 2 of 11 volunteers reported severe AEs, which is comparable with other human infection models. For example, in experimental malaria infections, volunteers generally experience three to four AEs, of which one would be severe for several days[15]. In typhoid infections ~50% of volunteers report severe symptoms and 55% experiences fever[16] and in cholera infection studies 40% of volunteers experience 1.6–8.0 liters of diarrhea[17]. We thus conclude that 20 cercariae may be the optimal dose that was both well tolerated and detectable. A relationship between dose and symptoms, as suggested by epidemiological data[18], cannot be

statistically confirmed with the current number of volunteers. Regardless of dose, all symptoms had resolved without sequelae at 12 weeks.

The follow up of volunteers in our study unequivocally showed that anti-adult worm IgM (100% seroconversion by week 6) or serum CAA (10 of 17 positive by week 6) are the earliest diagnostic markers currently available. This is in accordance with previous studies in travelers[19–21]. We found levels of serum CAA comparable to those in low endemic settings[22]. In nonhuman primate models these levels reflect 5–24 cercariae reaching adulthood[8,23]. Although serum CAA levels were more stable compared to urine CAA, the higher volume input of urine allows for more sensitive detection[24]. As suggested in previous studies, the urine POC-CCA rapid test was not suitable to detect very low intensity infection[25]. The main advantage of measuring serum CAA levels, as opposed to antibody detection, is the potential to follow up after treatment and confirm cure. In doing so, we found low cure rates with a single dose of 40 mg kg^{-1} praziquantel. Although a 60 mg kg^{-1} dose seemed more effective, the subsequent changes in pharmacokinetic and pharmacodynamic parameters need to be evaluated to conclude this with certainty. Because of the synchronous development of adult worms and the absence of reinfection in our model, the CHI design allows for screening of existing and new (stage-specific) anthelmintics.

The controlled schistosome infection model presented here clearly differs from infections in endemic settings, where doses are not controlled and infection occurs repeatedly. In addition, the single-sex infection lacks production of eggs that drive chronic regulatory and modified Th2 responses[26]. The presence of IgG to soluble egg antigen, indicates cross-reactive epitopes between eggs and worms. The induction of chemokines IP-10 and MIP-1β aligned with the increase in both antigen-specific $CD4^+$ T cell IFN-γ and Th2 cytokine production, respectively. The IFN-γ production contrasts with predominantly Th2 profiles in epidemiological studies, which may be driven by egg-related responses[26]. Although we detected increases in $FOXP3^+CD25^+CD4^+$ T cells, these mainly occurred in symptomatic volunteers and were found at a later timepoint than IFN-γ- or Th2 cytokine-producing T cells. This would be in line with the induction of regulatory T cells to prevent deleterious immune responses[27], different from the chronic regulation found in endemic infections. These important differences in antigen exposure and subsequent immunological responses between single-sex infections and endemic chronic egg production may limit the use of the model to study anti-fecundity effects. However, the immunological observations from our model are comparable to acute infection models in travelers and baboons, where a mixed Th1 and Th2 response also dominates[28,29]. Integrated data analysis revealed that acute schistosomiasis symptoms were particularly associated with antigen-specific Th2 cytokine production and circulating MIP-1β and IgM production, but could not be predicted at baseline. In contrast to volunteers with symptoms, those without symptoms predominantly showed IgG1 antibody responses. Similarly to what has been performed for other infectious diseases[30], a trial with repeated controlled infections could address whether these IgM or IgG1 antibodies have a protective effect. Currently, the clinical and immunological data from baseline alone cannot predict who will become symptomatic. However, the analysis of longitudinal responses reveals a clear profile predicting presence or absence of symptoms in 15 out of 16 participants. Unraveling the interplay between symptoms, immune responses and resistance to schistosome reinfection provides an opportunity for identification of new antigens for vaccine development[31].

Immunological models for protection against *S. mansoni* were developed in rodents and nonhuman primates by repeated exposure to radiation attenuated cercariae[23,32]. In humans, three candidate *S. mansoni* vaccines are currently in clinical development[6]. A phase I study with rSm14/GLA-SE showed a good safety profile and immunogenicity[7]. Phase I safety results for Sm-

TSP-2 are expected soon (ClinicalTrials.gov identifier: NCT02337855), while Sm-p80 is currently entering phase I testing on the basis of promising efficacy data in baboons[8]. The limited funding opportunities and large sample size required for phase III testing preclude testing of all three candidates in phase III trials. Despite the differences between chronic *S. mansoni* infection in the field and the controlled human *S. mansoni* infection, the model provides an opportunity to obtain preliminary efficacy data on these vaccines and reduce the costs by allowing selection of the most promising candidates, which may be co-formulated to maximize benefit[12].

Future vaccine studies aim at a 75% infection reduction in worm burden and egg output[10]. As there is a clear relationship between worm burden and serum CAA levels[24,33], we propose that the reduction in worm burden can be determined by measuring the median serum CAA level between week 7–12. Assuming an 80% power and $\alpha = 0.05$, this would require a group size of 11 individuals per arm. Currently the main limitation of our model is the use of male schistosomes only. Consequently vaccine targets that are more commonly expressed on females, such as the Sm-p80 vaccine candidate[8], cannot be fully evaluated. A female worm infection model would be of value to dissect mechanisms of action and sex-specificity of vaccines.

We conclude that this controlled human *S. mansoni* infection model results in an 82% infection rate with few severe side effects at a dose of 20 cercariae. In addition, this model provides insight into the onset of symptoms of a schistosome infection, the ensuing immune response and the performance of diagnostic tests over time. Notably, this model paves the way for cost-effective and rapid proof-of-concept testing of new vaccines and drugs.

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Methods

Study design

This phase I trial (ClinicalTrials.gov identifier: NCT02755324) was an open-label dose-escalation study at the Leiden University Medical Center (LUMC).

Male cercariae were produced as previously reported[34]. The initial dose was 10 cercariae (n = 3), which was escalated to 30 (n = 3) and subsequently de-escalated to 20 cercariae (n = 3) on the basis of AEs. The 20 cercariae infection was then validated in another group (n = 8). The viability of cercariae was confirmed by imaging of cercariae penetrating skin explants[35]. The cercariae were applied to the volunteer's forearm in 0.5–1 ml of water for 30 min, the number of remaining cercariae were counted by microscopy. Volunteers were observed for 30 min after exposure.

Volunteers were followed bi-weekly between week 0–24 and on week 52. During each visit, AEs were recorded. Symptoms of an acute schistosomiasis syndrome were defined as moderate to severe symptoms of malaise, fatigue, fever, night sweats, flu-like symptoms or headache, between 2–7 weeks after exposure. Safety reports were reviewed regularly by an external safety monitor, who advised on dose escalation. Blood and urine samples were collected at all visits.

The pre-patent period was defined as the time until serum CAA levels were above 1.0 pg ml^{-1} [24]. At week 12 all volunteers were treated with 40 mg kg^{-1} praziquantel in two doses. A second regimen of 60 mg kg^{-1} praziquantel in two doses was provided if serum CAA levels persisted 3–6 weeks after treatment. Cure was defined as serum CAA levels ≤0.5 pg ml^{-1}.

The study was approved by the LUMC Institutional Medical Ethical Research Committee (Institutional Review Board P16.111). It was performed according to the European Clinical Trial Directive 2001/20/EC, in accordance with ICH-GCP guidelines and the Declaration of Helsinki[36,37].

Informed consent procedure

Healthy 18–45-year-old *Schistosoma*-naïve volunteers were screened by medical history, general physical examination and safety laboratory tests. Informed consent was obtained from all volunteers.

Through advertisements, volunteers provided their email address and received written information. When they so wished, volunteers could schedule a screening visit at least 3 d after having received the information. They were then requested to complete an application form, which included a questionnaire regarding their health.

During the 1.5–2-h screening visit, the study purpose and procedures were explained and questions answered. The possible AEs and right of withdrawal were explained to the volunteers. The informed consent form was signed and a full physical exam was performed. All volunteers were required to consent to an HIV, hepatitis B (HBV) and hepatitis C (HCV) serological screening, urine toxicology and (for females), a pregnancy test at screening.

At the infection day (14 d to 23 weeks after screening), informed consent was reconfirmed, and a final check of inclusion and exclusion criteria was performed, including a focused physical exam. Volunteers were exposed to *male Schistosoma mansoni* cercariae after baseline assessment and safety laboratory tests.

The first volunteer was included on 27 October 2015 and the last volunteer was included on 1 February 2018.

All three volunteers gave permission to use the photographs (Fig. 2a–c) taken of their skin after cercarial exposure for publication.

Inclusion and exclusion criteria

Inclusion criteria

- Volunteer is aged ≥ 18 and ≤ 45 years and is in good health.
- Volunteer has adequate understanding of the procedures of the study and agrees to abide strictly thereby.
- Volunteer is able to communicate well with the investigator and is available to attend all study visits.
- Volunteer will remain within Europe (excluding Corsica) during the study period and is reachable by mobile telephone from week 3 to week 12 of the study period.
- Volunteer agrees to refrain from blood donation to Sanquin or for other purposes throughout the study period.
- For females: volunteer agrees to use adequate contraception and not to breastfeed for the duration of study.
- Volunteer has signed informed consent form.

Exclusion criteria

- Any history, or evidence at screening, of clinically significant symptoms, physical signs or abnormal laboratory values suggestive of systemic conditions, such as cardiovascular, pulmonary, renal, hepatic, neurological, dermatological, endocrine, malignant, hematological, infectious, immune-deficient, psychiatric and other disorders, which could compromise the health of the volunteer during the study or interfere with the interpretation of the study results. These include, but are not limited to, any of the following:
- body weight <50 kg or body mass index <18 or >30 kg m^{-2} at screening;
- positive HIV, HBV or HCV screening tests;
- the use of immune-modifying drugs within 3 months before study onset (inhaled and topical corticosteroids and oral anti-histamines exempted) or expected use of such during the study period;
- history of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years;
- any history of treatment for severe psychiatric disease by a psychiatrist in the past year;
- history of drug or alcohol abuse interfering with normal social function in the period of 1 year before study onset;
- any clinically significant abnormalities (including extended QT interval) on electrocardiogram.
- The chronic use of any drug known to interact with praziquantel, artesunate or lumefantrine (artesunate combined with lumefantrine served as alternative treatment of schistosomiasis in an earlier phase of infection) metabolism (for example, phenytoin, carbamazepine, phenobarbital, primidone, dexamethasone, rifampicin, cimetidine, flecainide, metoprolol,

imipramine, amitriptyline, clomipramine, class IA and III anti-arrythmics, antipsychotics, antidepressants, macrolides, fluorquinolones, imidazole- and triazole antimycotics and antihistamines). Because lumefantrine may cause extension of QT-time, chronic use of drugs with effect on QT interval are excluded from the study.
- For female volunteers: positive urine pregnancy test at screening
- Any history of schistosomiasis or treatment for schistosomiasis
- Positive serology for schistosomiasis or elevated serum or urine CAA at baseline
- Known hypersensitivity to or contraindications (including co-medication) for use of praziquantel, artesunate or lumefantrine
- Being an employee or student of the Department of Parasitology or Infectious Diseases of the LUMC.

Adverse events

Grading

- Mild (grade 1): awareness of symptoms that are easily tolerated and do not interfere with usual daily activity
- Moderate (grade 2): discomfort that interferes with or limits usual daily activity
- Severe (grade 3): disabling, with subsequent inability to perform usual daily activity, resulting in absence or required bed rest
- Serious AE: any untoward medical occurrence in a patient or trial participant, which does not have a causal relationship with the treatment, and:
    - is fatal, and/or
    - is life-threatening for the volunteer, and/or
    - makes hospital admission or an extension of the admission necessary, and/or
    - causes persistent or significant invalidity or work disability, and/or
    - manifests itself in a congenital abnormality or malformation, and/or
    - could, according to the person that carries out the research, have developed to a serious undesired medical event, but was, however, prevented due to premature interference.

Causality

Unrelated
- Not related: a relationship to the administration of *the S. mansoni* male cercariae cannot be reasonably established; another etiology is known to have caused the AE or is highly likely to have caused it.
- Unlikely related: a relationship to the administration *of S. mansoni* male cercariae is unlikely; however, it cannot be ruled out.

Related
- Possibly related: there is a potential association between the event and administration of the *S. mansoni* male cercariae; however, there is an alternative etiology that is more likely.

- Probably related: administration of the *S. mansoni* male cercariae is the most likely cause; however, there are alternative reasonable explanations, even though less likely.
- Definitely related: administration of the *S. mansoni* male cercariae is the cause; another etiology causing the adverse event is not known.

Parasitological assays

CAA was measured in serum and urine by the previously described upconverting phosphor lateral flow (UCP-LF CAA) assay[24,38]. The presence of urine CCA was determined by the POC-CCA (Rapid Medical Diagnostics). The *Schistosoma* PCR on feces was performed according to previous descriptions (ISO 15189:2012-certified)[39].

Antigen detection assays

Upconverting phosphor lateral flow assay for circulating anodic antigen
Both serum and urine were analyzed for schistosome CAA using a UCP-LF CAA assay. The assay was performed as described previously[24,38]. In brief, 500 µl of serum (or 4 ml of urine) samples or standards were diluted 1:1 in 4% trichloro-acetic acid (TCA) (or diluted 5:1 in 12% TCA for urine samples) and incubated for 5 min at room temperature. Serum samples were centrifuged for 10 min at 13,000 r.p.m. (and urine samples were centrifuged for 45 min at 4,000 r.p.m.). Then 500 µl of supernatants of serum (or 4 ml of urine) was applied to 0.5-ml (or 4.0-ml) Amicon filtration devices (Amicon Ultra-0.5 (or Amicon Ultra4), Millipore) and concentrated to approximately 20 µl by centrifugation for 25 min at 13,000 r.p.m. for serum (or 60 min at 4,000 r.p.m. for urine). The concentrates were diluted 1:5 in LF assay buffer and incubated in microtiter plate wells at 37 °C for 1 h while shaking. LF strips were inserted into the wells and incubated for 3 h or overnight, before being read on a modified Packard FluoroCount microtiter plate reader[24]. A TCA-soluble fraction of *S. mansoni* adult worm antigen with known CAA concentration was used as a reference standard for the quantification of the antigen. Predefined cutoff values were used, where a serum CAA concentration above 1 pg ml^{-1} was defined as positive, below 0.5 pg ml^{-1} as negative and between 0.5 and 1.0 as undecisive[24].

Point-of-care circulating cathodic antigen
Urine samples were tested for the presence of schistosome CCA using a commercially available rapid diagnostic test (POC-CCA, batch no. 170622073, Rapid Medical Diagnostics) according to the manufacturer's procedure. Readings above a trace line were considered as positive.

Immune responses

Clinical diagnostics

Schistosome-specific antibodies, IgM against adult worms and anti-SEA IgG were determined according to ISO 15189:2012-certified routine diagnostic in-house IFA and ELISA respectively, which have been in use for the clinical diagnosis of schistosomiasis at the LUMC for decades[40,41].

IgM adult worm antibodies by IFA

IgM antibodies against adult worms were detected by an in-house IFA assay as previously described[42]. This IFA, as well as the ELISA described below, are currently in use at LUMC as the routine antibody detecting assays for the diagnosis of imported schistosomiasis and feature in all laboratory quality assessment requirements (ISO 15189:2012-certified), including successful participation in an external quality assessment scheme (UK-NEQAS). In brief, sections of Rossman's fixed male adult worms were incubated with a twofold dilution series of serum samples starting at 1:8 dilution. Following incubation with goat anti-human IgM (u-chain specific)-FITC antibody (Sigma-Aldrich; F5384), slides were examined using a fluorescence microscope. A negative control and a positive reference serum were run in parallel at each slide. The titer was determined as the dilution of the sample at which the fluorescence of adult worm gut epithelium was still visible. Samples were considered positive if titers were above 1:8. Previous studies showed that the IgM detected in this IFA is mainly directed against CCA[42].

IgG against soluble egg antigen

IgG antibodies directed against *S. mansoni* SEA were detected by a previously described in-house ELISA with some minor modifications[40]. Crude SEA was prepared from *S. mansoni* eggs collected from the livers of infected hamsters[43,44]. A concentration of 5 µg protein ml^{-1} was diluted in 100 µl of 0.1 M sodium carbonate buffer (pH 9.6) and coated overnight at 4 °C in 96-well plates (Polysorb NUNC). Plates were stored at −80 °C until use. After thawing, plates were washed and blocked in 1% BSA in PBS for 1 h at 37 °C. A dilution series of serum samples from 1:16 to 1:2,048 in a solution with 5% FCS and 0.05% Tween in PBS were incubated for 1 h at 37 °C. Plates were washed and incubated for 1 h at 37 °C with mouse anti-human IgG alkaline phosphatase 1:10,000 (Sigma-Aldrich; A2064) in 4% BSA and 0.05% Tween/PBS. Para-nitrophenyl phosphate (pNPP) substrate (Sigma-Aldrich; P5994) in 0.1 M diethanolamine buffer (pH 9.6) (Merck) was added for 1 h at room temperature after washing. Plates were read with the Multiskan EX reader at 405 nm. A negative control and a positive reference serum were run in parallel at each plate. The titer was determined as the dilution of the sample at which the extinction is higher or equal to the reference standard. Samples were considered positive if titers were above 1:16.

Experimental immunological assays

Serum was analyzed for total IgE, *S. mansoni* AWA-specific IgE and IgG by ELISA[45–47]. Data were expressed as arbitrary units (AU ml^{-1}). Peripheral blood mononuclear cells (PBMCs) were evaluated for their phenotype and function after 24-h stimulation with crude *S. mansoni* AWA by flow cytometry.

Adult worm antigen-specific IgE, IgG, IgG1 and IgG4

S. mansoni AWA-specific IgE and IgG was measured by ELISA modified from previous protocols[45,46]. In brief, adult *S. mansoni* worms were collected from hamsters and crude AWA was prepared as described previously[48]. MaxiSorp plates (Nunc) were coated overnight with 5 µg ml^{-1} AWA diluted in carbonate buffer pH 9.6. After blocking with 5% BSA/PBS, the sera were diluted with a solution with 5% FCS and 0.05% Tween in PBS and the presence of IgG1 or IgG4 was shown by using horseradish peroxidase (HRP)-labeled anti-human IgG1 or HRP-labeled anti-human IgG4 (1:3,000 dilution; Sanquin). For measuring total anti-AWA IgG antibody, alkaline phosphatase-conjugated anti-human IgG (1:5,000 dilution; Sigma) was added, whereas for the anti-AWA IgE

assay, the plate was incubated with biotinylated goat anti-human IgE (1:1,000 dilution; Vector Laboratories) followed by streptavidin HRP conjugate (1:10,000 dilution; Sanquin). IgG1 and IgG4 assays were developed using tetramethylbenzine, stopped with 10% H_2SO_4 and the absorbencies were measured at 450 nm. For total IgG and IgE, the color was developed by addition of pNPP (Sigma) diluted in diethanolamine buffer and optical density was measured at 405 nm. For all four assays, the levels of antibody present in a given sample were expressed in AU ml^{-1} or International Units (IU ml^{-1}) according to the standard curve of pooled sera from inhabitants of an *S. mansoni* endemic area in Ghana. The levels of antibody present in a given samples were expressed in AU ml^{-1} for total IgG, IgG1 and IgG4 or in IU ml^{-1} for IgE. Seroconversion was defined as antibody levels above 2× s.d. of the baseline.

Total IgE

Total IgE levels were measured as previously described[47]. Briefly, MaxiSorp plates were coated overnight with rabbit anti-human IgE (Dako). Plates were blocked with PBS 5% BSA followed by incubation of diluted samples in PBS 0.05% Tween-20. As a reference, the World Health Organization standard of human serum IgE (NIBSC) was used, starting at a concentration of 90 IU ml^{-1}. After a washing step, the plates were incubated with IgE biotinylated goat anti-human IgE antibody (Vector Laboratories) followed by an incubation with streptavidin alkaline phosphatase conjugate (Boehringer Mannheim). The color was developed by addition of pNPP (Boehringer Mannheim) diluted in diethanolamine buffer and optical density was measured at 405 nm. The results were expressed in IU ml^{-1}. Seroconversion was defined as antibody levels above 2× s.d. of the baseline.

Ex vivo cytokines

Serum samples were tested for the presence of different cytokines using a commercially ProcartaPlex Multiplex Immunoassay (17-plex, lot 178863000, Invitrogen) according to the manufacturer instructions. The following cytokines were measured: IFN-α, IFN-β, IL-1β, IL-10, IL-12p70, IL-13, IL-15, IL-2, IL-22, IL-23, IL-4, IL-6, IP-10, MCP-1, MIP-1α, MIP-1β and TNF-α using the Bioplex 200 Luminex (Bio-Rad).

Cellular immunology

Between week 0 and 24 after exposure every 4 weeks, human PBMCs were isolated from whole blood collected in heparin. Cells of two heparin tubes were diluted at least 1:2 with HBSS (ThermoFisher) at room temperature. Ten milliliters of ficoll at room temperature was added, followed by 25 min centrifugation at 400g with low brake. Cells were collected and washed with HBSS, counted and frozen in 10% DMSO, in RPMI Hepes (Invitrogen), with 100 U ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, 1 mM pyruvate/2 mM glutamate and 10% FCS (Bodinco). Subsequently, PBMCs were thawed in RPMI Hepes, with 100 U ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, 1 mM pyruvate/2 mM glutamate and 10% FCS and rested overnight at 37 °C with 5% CO_2. Cells were counted and transferred to a 96-well round bottom plate (Corning) with 500,000 cells per well. Cells were stimulated with AWA (50 µg ml^{-1}) for 24 h. Staphylococcal enterotoxin B (SEB) (Sigma-Aldrich) 200 ng ml^{-1} was used as a positive control and RPMI Hepes (Invitrogen), with 100 U ml^{-1} penicillin, 100 µg $m^{l-}1$ streptomycin, 1 mM pyruvate/2 mM glutamate and 10% FCS as a negative control. After 4 h of incubation 5 mg ml^{-1} brefeldin A (Sigma) was added to SEB-stimulated wells and after 20 h to AWA- and medium-stimulated wells. After a total stimulation of 24 h, the cells were stained with Aqua (Invitrogen) and fixed with 3.9% formaldehyde (Sigma). After fixating,

the cells were stained with the following antibodies: CD3, CD4, IFN-γ, IL-2, Th2-cytokines (IL-4, IL-5, IL-13), TNF and IL-10 (Supplementary Table 4). Human FC block was used to avoid nonspecific interactions. The cells were measured with the FACSCanto II (BD Biosciences; Supplementary Fig. 1). The data were analyzed with FlowJo 10.5 software for MAC OS. The gating was placed with the help of fluorescence minus one controls, the medium as a negative control and SEB as a positive control. The leftover, aqua-stained and fixed cells were frozen in 10% DMSO, in RPMI Hepes (Invitrogen), with 100 U ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, 1 mM pyruvate/2 mM glutamate and 10% FCS (Bodinco) and stored at −80 °C. The cells were thawed at 37 °C and stained with the following antibodies: CD3, CD4, CD25, CD127 and FOXP3 (Supplementary Table 4). As before, human FC block was used to avoid nonspecific interactions. The cells were measured with the FACSCanto II (BD Biosciences; Supplementary Fig. 1). The data were analyzed with FlowJo 10.5 software for MAC OS. The gating was placed with help of fluorescence minus one controls, the medium as a negative control and SEB as a positive control.

Statistical analysis

All 17 volunteers were included in the intention-to-treat analysis (safety analysis and parasitological assays). One volunteer was excluded from the per-protocol analysis (all immunological readouts) on the basis of high baseline AWA-specific IgG and IgG1 levels. Samples from individual volunteers were measured once and plotted as single values.

Demographics and the presence of symptoms between groups were analyzed with a Mann–Whitney *U*-test, time to patency with a log-rank test and correlations with a Spearman's rank test. Changes in the frequency of cytokine-producing cells over time were analyzed using a linear mixed model. Time was considered as the fixed effect and the volunteer ID as a random effect for the intercept. *P* values based on Student's *t*-tests were obtained using the Satterthwaite's degrees of freedom method. In the cytokine boxplots, the negative values (after subtracting the medium condition) were set to zero to prevent a negative cytokine response. However, the statistical analysis was performed on the unaltered data. All statistical tests were two-tailed with α set at the 0.05 level.

Analyses were performed using IBM SPSS statistics for Windows, v.23.0 (IBM Corp.) and R (v.3.5). The R packages used were ggplot2, lme4 and lmerTest[49–51].

P values were considered significant when $P < 0.05$.

MixOmics and data integration

Data integration was performed using the mixOmics package in R (v.6.8.0)[52,53]. This method allowed us to correlate across datasets, while associating features with outcome. Feature selection was performed using Lasso-like penalization for each of the datasets. The number of components was set at two and tuning was performed to find the minimum number of features needed per dataset (in a range from 1–3 per component) and the correlation between datasets was entered into the design matrix. The number of features included in the final model was 3, 3, 2 and 5 for cytokines, antigens, antibodies and cellular responses, respectively. A correlation of 0.75 between datasets was used in the design matrix.

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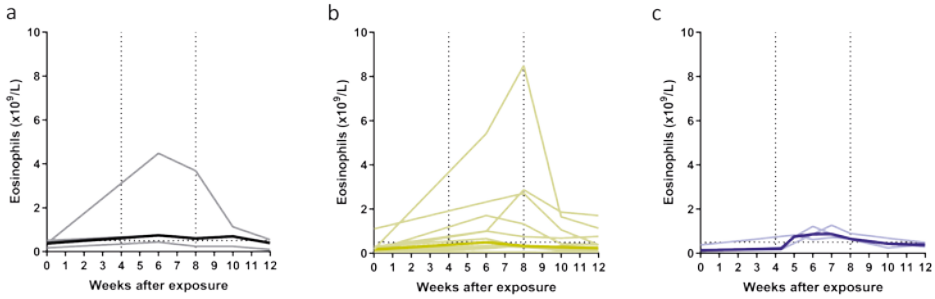
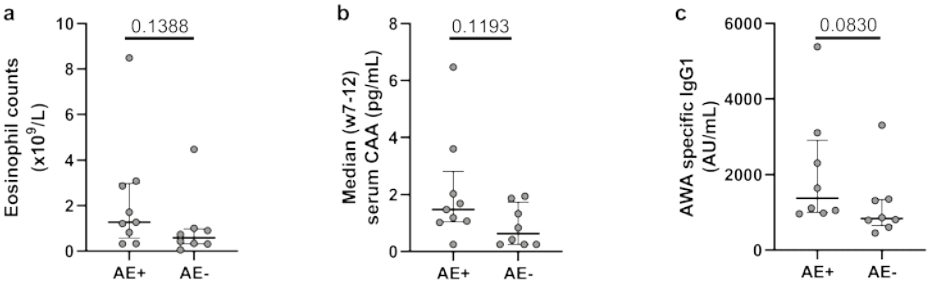
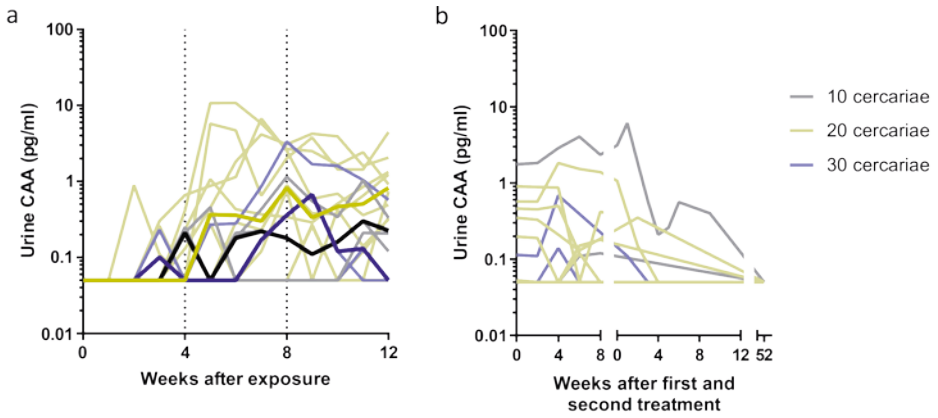
Extended data



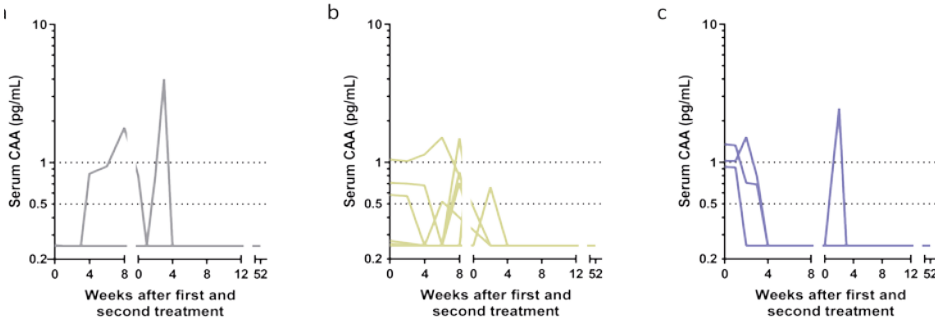
Extended Data Figure 1. Eosinophil counts. a-c. Eosinophil counts (x10^9/L) per volunteer and the median per group. The thin green, red, and blue lines represent data of individual volunteers infected with 10 (*n* = 3), 20 (*n* = 11) or 30 (*n* = 3) cercariae respectively, while thick lines represent the median of each group.



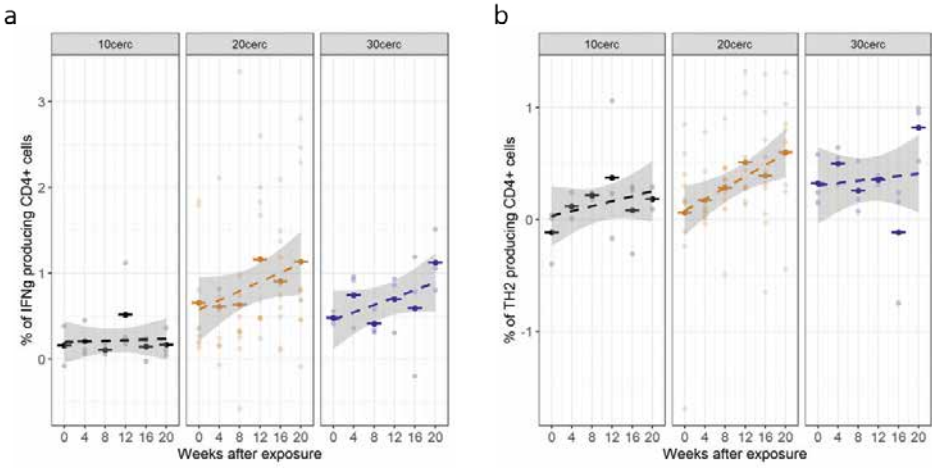
Extended Data Figure 2. Relation between symptoms of an acute schistosomiasis syndrome and immunological readouts. The relation between the presence of symptoms of an acute schistosomiasis infection and **a**. the highest eosinophil count (*n* = 17), **b**. the median serum CAA level from week 7 to 12 (*n* = 17), or **c**. the AWA specific IgG1 response at week 16 (*n* = 16). All using the two-sided Mann-Whitney *U* test. Individual data is presented as dots, the line represents the median, while the error bars represent the interquartile range of the groups.



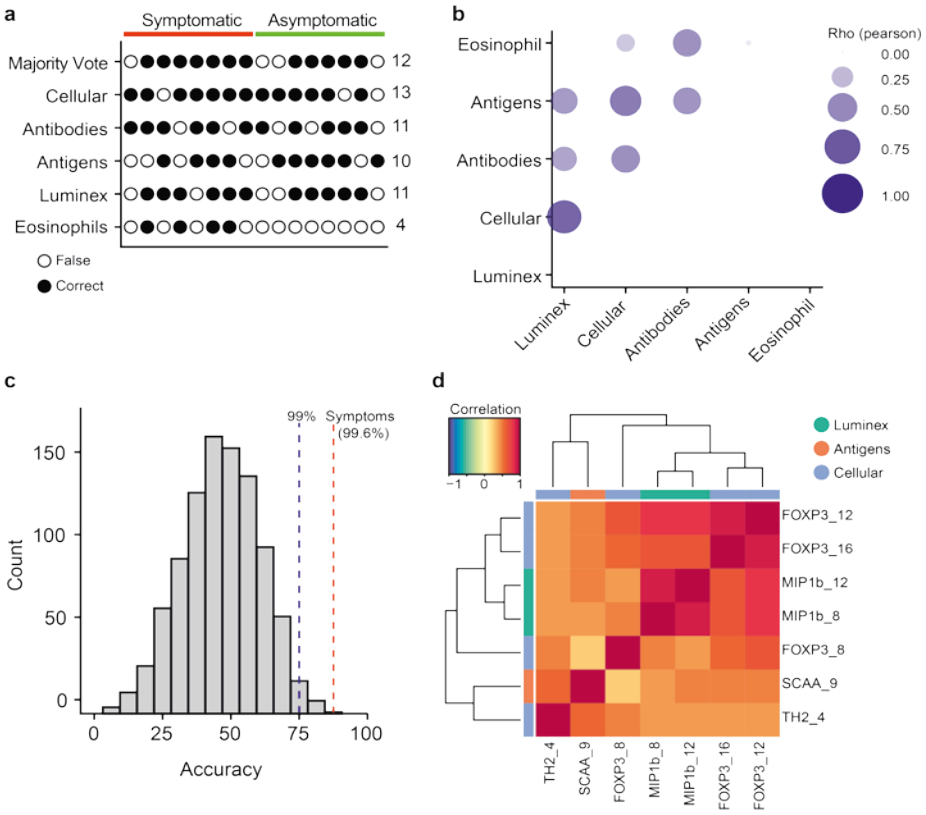
Extended Data Figure 3. Urine CAA levels. a. Urine CAA levels after exposure and **b**. after first and second praziquantel treatment (week 0) and at week 52 after exposure. The thin green, red, and blue lines represent of individual volunteers infected with 10 (*n* = 3), 20 (*n* = 11) or 30 (*n* = 3) cercariae respectively, while thick lines represent the median of each group before treatment.



Extended Data Figure 4. Serum CAA levels after treatment. a-c. Serum CAA levels in pg/ml after the first treatment, second treatment, and at week 52. All values below the detection threshold of 0.5 pg/mL, are plotted at 0.25 pg/mL. The gray, yellow and blue lines represent data of individual volunteers infected with 10 (*n* = 3), 20 (*n* = 11) or 30 (*n* = 3) cercariae respectively.

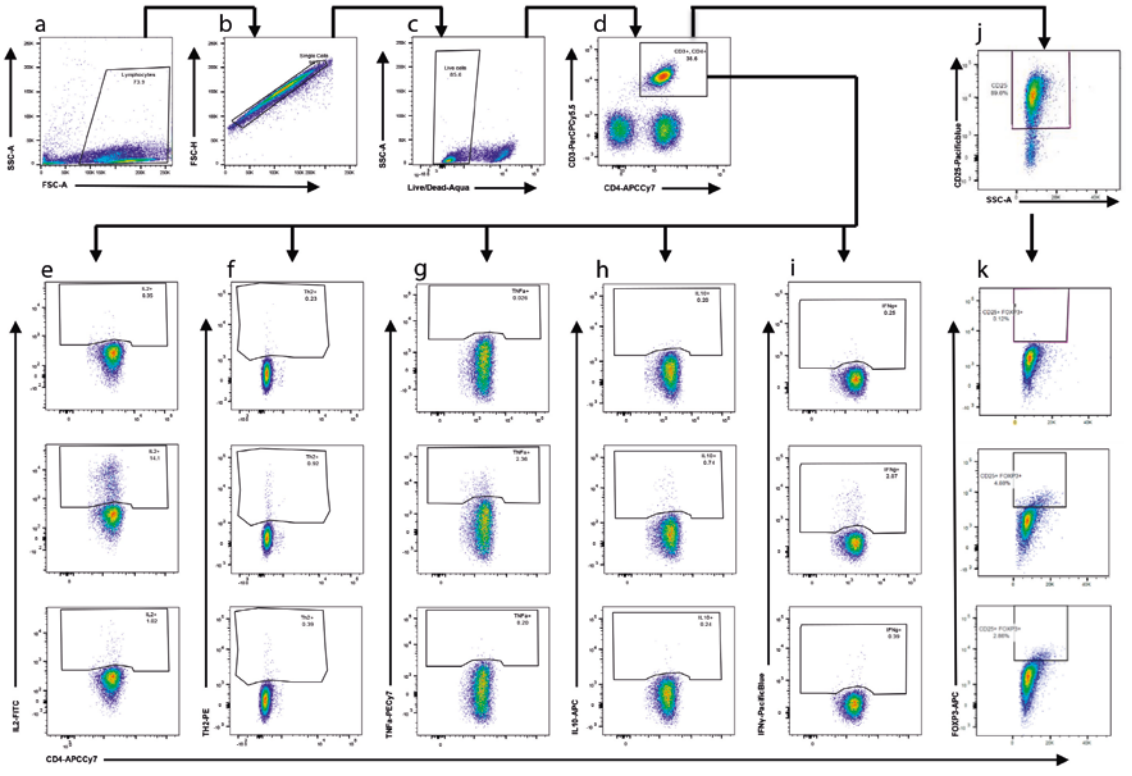


Extended Data Figure 5. IFN-γ and TH2 cytokine producing CD4⁺ T-cells over time. The percentage of **a**. IFN-γ and **b**. Th2-cytokine producing CD4⁺ T-cells over time in weeks after exposure in the 10 cercariae (gray, *n* = 3), 20 cercariae (yellow, *n* = 10) and 30 cercariae (blue, *n* = 3) groups. Dotted lines are linear regression lines, gray areas are confidence intervals, light dots are individual data, and horizontal lines with dots are the average values.



Extended Data Figure 6. Effect of eosinophil data and model performance. a. Individual predictions across folds for the full model per dataset including majority vote. Each symbol represents one prediction, with volunteers in columns and datasets in rows. Filled circles indicate a correct prediction and open circles a false prediction. Symptomatic and asymptomatic volunteers are indicated in red and the number of correct predictions per dataset is indicated. **b**. Mean Pearson correlation score between datasets using the first component of the projection onto the latent space across all folds from the model including all datasets. Size and color of circles reflect the mean rho value. **c**. Permutations analysis (n = 1000) with leave-one-out cross-validation on the full model using all subjects and including the four datasets without eosinophils. Blue and red dashed lines indicate the 99th percentile and the accuracy when comparing symptomatic and asymptomatic individuals (99.6%), respectively. **d**. Spearman correlation matrix of the seven consensus features selected in > 75% of folds in the leave-one-out cross-validation. Features were clustered using hierarchical clustering with complete linkage on Euclidean distance. All graphs are based on n = 16.

Supplementary



Supplementary Figure 1. Flow cytometry gating strategy. Gating of stimulated PBMCs. **a**. gating on lymphocytes. **b**. gating on single cells. **c**. gating of live cells **d**. gating on $CD4^+$ cells. **e-i**: gating on selection of IL2, Th2 (IL-4+IL-5+IL-13), TNF-α, IL10, or IFN-γ positive cells. **j**. gating on $CD25^+$ cells **k**. gating on FOXP3 positive cells. For cells stimulated with either medium, Staphylococcal Enterotoxin B (SEB) or adult worm antigen (AWA).

Supplementary table 1. Demographics and cercariae retrieved after exposure. Demographics of included volunteers, (median and range). The number of heads, tails and whole body cercariae retrieved from volunteers' skin after infection (median and range). The 10, 20 and 30 cercariae groups were compared using the two-sided Mann-Whitney U test (top) or the Spearman's rank correlation (bottom).

	10 cercariae (*n*=3)	20 cercariae (*n*=11)	30 cercariae (*n*=3)	All volunteers (*n*=17)	*P*-value
Demographics					
Gender					0.34
• Male	1	5	0	6	
• Female	2	6	3	11	
Age in years (median (range))	20 (19-26)	30 (18-45)	23 (21-25)	25 (18-45)	0.21
BMI in kg/m^2 (median (range))	21.7 (20.5-24.1)	25.1 (20.7-28.5)	20.9 (18.6-24.4)	24.1 (18.6-28)	0.12
Cercariae retrieved after exposure					
Heads (median (range))	0 (0-1)	0 (0-1)	1 (0-1)	0 (0-1)	0.37
Tails (median (range))	5 (4-6)	8 (4-12)	14 (10-19)	8 (4-19)	**0.002**
Whole body cercariae (median (range))	0 (0-1)	0 (0-3)	1 (0-3)	0 (0-3)	0.33

Supplementary table 2. Volunteer's age and sex. Listing of volunteer code, group allocation, cercarial exposure, sex and age.

Volunteer	Group	Cercarial exposure	Sex	Age
Cs1-501	group 1	10	F	20
Cs1-516	group 1	10	F	26
Cs1-753	group 1	10	M	19
Cs1-106	group 2	30	F	23
Cs1-495	group 2	30	F	21
Cs1-674	group 2	30	F	25
Cs1-069	group 3	20	F	22
Cs1-229	group 3	20	F	35
Cs1-425	group 3	20	M	20
Cs1-004	group 4	20	M	18
Cs1-102	group 4	20	F	30
Cs1-464	group 4	20	M	36
Cs1-759	group 4	20	M	25
Cs1-780	group 4	20	F	39
Cs1-832	group 4	20	F	23
Cs1-923	group 4	20	M	35
Cs1-967	group 4	20	F	45

Supplementary table 3. Adverse events. The number of volunteers developing a related (either possibly, probably or definitely related) solicited or unsolicited adverse event (AE) throughout the study period. Data is presented per cercarial dose group and in total. The maximum severity of an AE is presented in the table.

			10 cercariae (n=3)			20 cercariae (n=11)			30 cercariae (n=3)			Total (n=17)		
			Mild	**Moderate**	**Severe**	**Mild**	**Moderate**	**Severe**	**Mild**	**Moderate**	**Severe**	**Mild**	**Moderate**	**Severe**
Local	**solicited**	Pruritus	3			10			2			15 (88%)		
		Cercarial dermatitis	3			10			3			16 (94%)		
	unsolicited	Burning feeling				3						3 (18%)		
		Dysesthesia				1						1 (6%)		
Systemic	**solicited**	Fever	1				1	1			2	1 (6%)	1 (6%)	3 (18%)
		Headache				1	4	1			3	1 (6%)	4 (24%)	4 (24%)
		Fatigue					3						3 (18%)	
		Malaise					2			1			3 (18%)	
		Cough					1						1 (6%)	
		Myalgia				1			1			2 (12%)		
		Night sweats					1						1 (6%)	
		Nausea		1							1		1 (6%)	1 (6%)
		Abdominal pain	1						1			2 (12%)		
		Diarrhea		1						1			2 (12%)	
	unsolicited	Less ability to focus	1									1 (6%)		
		Elevated liver enzymes												1 (6%)
		Syncope									1			1 (6%)
		Periorbital edema				1				1		1 (6%)	1 (6%)	
		Pharyngitis/sore throat				2						2 (12%)		

None of the volunteers experienced vomiting, urticaria, backpain or anorexia

Supplementary table 4. The antibodies used for staining of the cells.

Antibody	Label	Dilution	Company	Cat.
IL-2	FITC	1:25	BD Biosciences	340448
IL-4	PE	1:20	BD Biosciences	340451
IL-5	PE	1:250	Biolegend	504303
IL-13	PE	1:100	Biolegend	501903
CD4	PerCP-ef710	1:400	eBioscience	46-0047
TNF	PE-Cy7	1:1000	eBioscience	25-7349
IL-10	APC	1:250	BD Biosciences	554707
CD3	APC ef780	1:800	eBioscience	47-0038
IFN-γ	BV421	1:1000	Biolegend	502531
CD3	FITC	1:100	BD Biosciences	555339
CD4	Pe-Cy7	1:150	BD Biosciences	557852
CD25	BV421	1:200	BD Biosciences	562442
CD127	APCe780	1:200	eBioscience	47-1278-42
FOXP3	APC	1:100	eBioscience	17-4776-42
Human FC block	-	1:100	eBioscience	14-9161
Live/Dead Aqua	AmCyan	1:400	ThermoFisher	L34957