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ORIGINAL ARTICLE

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Antibody responses to *Schistosoma mansoni* schistosomula antigens

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Summary

While antigens from *Schistosoma* schistosomula have been suggested as potential vaccine candidates, the association between antibody responses with schistosomula antigens and infection intensity at reinfection is not well known. *Schistosoma mansoni*-infected individuals were recruited from a schistosomiasis endemic area in Uganda (n = 372), treated with 40 mg/kg praziquantel (PZQ) and followed up at five weeks and at one year post-treatment. Pre-treatment and five weeks post-treatment immunoglobulin (Ig) E, IgG1 and IgG4 levels against recombinant schistosomula antigens rSmKK7, rSmLy6A, rSmLy6B and rSmTSP7 were measured using ELISA. Factors associated with detectable pre-treatment or post-treatment antibody response against the schistosomula antigens and the association between five-week antibody responses and one year post-treatment reinfection intensity among antibody responders were examined. Being male was associated with higher pre-treatment IgG1 to rSmKK7, rSmLy6a and AWA. Five weeks post-treatment antibody responses against schistosomula antigens were not associated with one year post-treatment reinfection intensity among antibody responders' antibody levels against rSmKK7, rSmLy6B and rSmTSP7 dropped, but increased against rSmLy6A, AWA and SEA at five weeks post-treatment among antibody responders. *S. mansoni*-infected individuals exhibit detectable antibody responses to schistosomula antigens that are affected by treatment. These findings indicate that schistosomula antigens induce

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highly varied antibody responses and could have implications for vaccine development.

KEYWORDS
antibody responder, IgE, reinfection, *Schistosoma mansoni*, schistosomula antigens

1 | INTRODUCTION

Approximately 206 million people worldwide required treatment for schistosomiasis in 2016.[1] Control programmes in affected countries have reduced the morbidity associated with schistosomiasis,[2] yet despite this, treatment is unable to prevent reinfection with *Schistosoma* species, and consequently, transmission of schistosomiasis remains a global problem. Mass drug administration with praziquantel (PZQ) alone may not ultimately control schistosomiasis,[3] and therefore, a solution such as a prophylactic vaccine is needed to provide long-term immunity and preferably complement the PZQ-based schistosomiasis control strategy.[4] There is currently no vaccine against schistosomes, and only a few vaccine candidates are in clinical trials. Despite the limited success, defined antigens continue to be investigated as vaccine targets. Among these are those from the larvae of the schistosome, known as schistosomula. The schistosomula develop when free-living cercariae penetrate host skin, lose their bifurcated tails and shed their cercarial glycocalyx coat in the skin in a process called transformation. Early work on schistosome parasite biology showed that transformation makes the skin schistosomula vulnerable to killing by host immune responses.[5] This killing is mediated through complement fixation [6,7] and antibody (IgE, IgA and IgG)-dependent cell-mediated cytotoxicity (ADCC).[8-10] The antibodies coat the schistosomula and allow eosinophils to kill the parasite in vitro.[11,12] This suggests that the schistosomula are a potential source of vaccine candidates.

By examining the *S. mansoni* transcriptome, genes that are highly expressed or upregulated during the schistosomula stage compared to the infective cercariae can be identified.[13] Indeed, DNA microarray-based analysis of the *S. mansoni* life cycle has revealed schistosomula-enriched gene products, such as those coding for SmKK7 (smp_194830), *S. mansoni* lymphocyte antigen 6 isoforms A and B (SmLy6A; smp_019350, SmLy6B; smp_105220) and *S. mansoni* tetraspanin 7 (SmTSP7; smp_099770).[14] SmLy6A and SmLy6B are probably glycophosphatidylinositol (GPI)-anchored antigens, found in schistosomula,[13,15] but also in adult tegumental and mesenchymal tissues.[16,17] Although SmLy6A and SmLy6B are homologues of human CD59, which inhibits the formation of the complement membrane attack complex, they do not inhibit complement fixation and their function remains unknown.[17] SmKK7 is a putative immunomodulatory protein found in the peripheral nervous system of *S. mansoni* adults [18] and upregulated in 7- and 14-day schistosomula.[15] SmTSP7 is a membrane-spanning tetraspanin with unknown function but has been identified in the tegument of the adult worm.[19]

Epidemiological studies have shown that specific host antibodies to *Schistosoma* antigens contribute to immunity to schistosomiasis. For instance, antibody responses targeting *S. mansoni* adult worm antigens are associated with resistance or susceptibility to reinfection of people at risk in endemic areas.[20,21] Although adult worm antigen-specific IgE is associated with resistance against reinfection, it is important to note that vaccine candidates that induce IgE production may not be safe for use, particularly in previously helminth-infected individuals. This was accentuated during a trial involving a hookworm vaccine candidate that was discontinued when previously hookworm-infected participants developed an IgE-mediated allergic reaction.[22,23] The same hookworm vaccine had earlier been shown to be safe with healthy hookworm-naive individuals.[24] This suggests that helminth (including *Schistosoma*) antigens should be pre-clinically screened using sera from infected people to determine whether antigen-specific IgE is present.

On the other hand, IgG4 blocks IgE-mediated protective immunity by competing with IgE to bind shared epitopes [25] and engaging the inhibitory IgG receptor, FcγIIb, that downregulates signalling from the IgE receptor, FcεRI, on effector cells.[26,27] As a result, activation of effector cells is inhibited [28] and protective responses may be less effective. Indeed, *Schistosoma*-infected people, especially children (who are the most susceptible to reinfection), produce high levels of IgG4.[29,30] Although antibody responses (IgE and IgG) to crude purified extracts of schistosomula have been shown to contribute to human resistance,[31,32] few studies have looked at antibody responses to recombinant schistosomula antigens.[33]

The aim of this study was to determine how infection intensity, age and sex affect antibody responses to the recombinant *S. mansoni* schistosomula antigens rSmLy6A, rSmLy6B, rSmKK7 and rSmTSP7 in an endemic population. We also analysed the effect of treatment on the antibody responses against these recombinant antigens and the correlation between the antibody and cytokine responses (in the companion paper). Finally, we examined whether pre- or five weeks post-treatment antibody responses were associated with reinfection one year later.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

Informed consent was obtained from adults in Namoni to participate in this study. Children gave written assent to participate in the study. The Makerere University School of Biomedical Sciences Higher Degrees Research and Ethics Committee (reference number SBS

300) and the Uganda National Council for Science and Technology (reference number HS 1040) approved this work.

2.2 | Recruitment of study participants

Individuals were recruited from Namoni village, a Ugandan fishing community that is endemic with schistosomiasis at the shore of Lake Victoria as part of TheSchistoVac study (http://www.theschistovac.eu) to develop antigens for a prophylactic schistosomiasis vaccine. A total of 372 individuals aged between 6 and 40 years were recruited from Namoni (Figure 1). TheSchistoVac cohort in Namoni has been described elsewhere.[34] In September 2011, the participants were treated with two doses of praziquantel (40 mg/kg) one week apart and followed up for 5 weeks and a year. Infection intensity was determined from stool samples collected pre-treatment, at 5 weeks for efficacy of the PZQ treatment and one year for reinfection. Participants were asked to donate a venous blood sample immediately before the first praziquantel treatment and a further blood sample five weeks later. Plasma samples were separated, stored and later tested for antibody responses.

Of those recruited and followed up to the end of the study, 244 (65.6%) completed the study. However, 240 (64.5%) of those recruited had complete data on infection intensity, age, sex and levels of IgG1, IgG4 and IgE to AWA, SEA and the antigens at baseline, five weeks and one year after PZQ treatment (Figure 1).

The water contact behaviour of the study participants was obtained and recorded before treatment and included the duration of contact (time spent in the lake) and whether or not the participants bathed, swam, played, fished or processed fish, washed utensils or clothes in the lake, or engaged in transport across the lake or engaged in farm irrigation using lake water. The water contact behaviour was self-reported during questionnaire interviews.

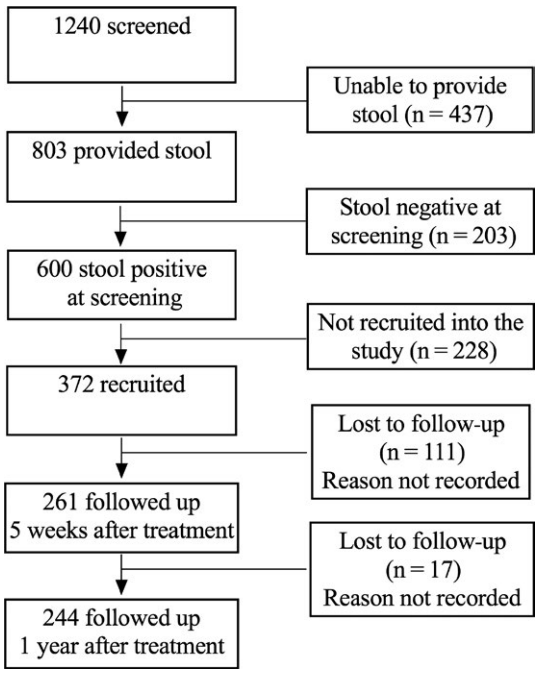

FIGURE 1 The study profile describing the screening, recruitment and follow-up of the study participants

2.3 | Stool examination by microscopy

Before treatment, three stool samples were collected on three consecutive days in the morning and two thick smears made from each sample. The six slides were examined using the Kato Katz method for the number of *S. mansoni* eggs as previously described.[35] The egg count was the average of the six slides from the three samples. The infection intensity was calculated by multiplying the average egg count with 24 and expressed as egg count per gram of stool. The infection intensity of the participants was classified as either light (1-99 epg), moderate (100-399 epg) or heavy (>400 epg) based on the WHO criteria.[36] Additional stool samples were collected five weeks and one year after PZQ treatment. Of the 240 infected individuals, 186 (77.5%) had no eggs (putatively cured)[37] by the Kato Katz method performed on three stool samples five weeks after PZQ treatment. One year after PZQ treatment, 154 (82.8%) of those cured were egg positive, determined by the Kato Katz method performed on six smears from three stool samples. Reinfection was defined as the presence of eggs at one year after PZQ treatment.[38]

2.4 | *Schistosoma mansoni* antigens

The antigens used in this study were *S. mansoni* adult worm (AWA) and egg antigens (SEA) and recombinant schistosomula antigens rSmKK7, rSmLy6A, rSmLy6B and rSmTSP7. These antigens were identified as highly expressed products in the schistosomula life cycle stage after screening the *Schistosoma* transcriptome using DNA microarrays, as previously described.[14,16] Specifically, a >5-fold increase in expression when comparing normalized expression averages from snail (egg, miracidia, mother sporocyst and daughter sporocyst) to schistosomula (3-h, 24-h, 3-day and 6-day schistosomula) life stages was observed.[16] Recombinant antigens were expressed in *Escherichia coli* and purified using methods as previously described for rSmKK7 (smp_194830),[14] rSmLy6A and rSmLy6B.[15] For SmTSP7, 78 amino acids representing the extracellular loop 2 of the protein (108-185AA; defined by TMHMM2.0 software [39] were expressed using the same vector, expression parameters and purification methods as SmLy6A and SmLy6B. The selected proteins used in this study were screened for in silico similarity to known allergens, and any with a similarity were discarded from the pipeline. The selected proteins were further screened for IgE reactivity using sera from a schistosomiasis endemic cohort, Musoli. It was found that Musoli residents had minimal IgE reactivity to these proteins, and the antigens were therefore selected for further study.

2.5 | Blood collection and estimation of antibody responses

Plasma samples were separated from whole blood before and five weeks after PZQ treatment by centrifugation and stored at -20°C

TABLE 1 Baseline demographics of the study participants (N = 240)

Factor	Level	n (%)
Sex	Female	135 (56)
Age (years)	6-9	74 (31)
	10-13	76 (32)
	>14	90 (37)
Pre-treatment infection intensity (epg)	Light (1-99)	44 (18)
	Moderate (100-399)	41 (17)
	Heavy (>400)	155 (65)

until ready for use. The concentrations of IgG1, IgG4 and IgE specific for the schistosomula antigens (rSmKK7, rSLy6A, rSmLy6B, rSmTSP7) and the crude parasite antigens (AWA and SEA) were quantified using ELISA as previously described.[40] Briefly, the well surfaces of 384-well Microlon 600 high-binding plates (Greiner, Meadville, PA, USA) were washed with distilled water and coated overnight at 4°C with 15 μL/well of antigen diluted in sodium bicarbonate solution. The saturating concentrations of rSmKK7, rSmLy6A, rSmLy6B, rSmTSP7, AWA and SEA used were 13.40, 6.25, 9.60, 6.25, 8 and 1.25 μg/mL, respectively. The plates were blocked for 1 h, and sera from the infected Ugandan individuals and uninfected nonendemic European control samples were diluted 1/200 for IgG1 and IgG4 and 1/20 dilution for IgE, plated and incubated overnight at 4°C. The serum samples were tested in duplicate. Diluted monoclonal mouse anti-human IgG1, IgG4 and IgE biotin (Pharmingen, San Diego, CA, USA) were used and incubated at room temperature for 2 h for IgG1 and IgG4 or +4°C overnight for IgE, respectively. Detection was performed using Poly-HRP streptavidin complex (Mast Group, UK) and OPD substrate solution (Sigma, Ronkonkoma, NY, USA). The reaction was stopped using 2M sulphuric acid and absorbance read at test wavelength 490 nm and reference wavelength 630 nm on a PowerWave HT microplate reader (BioTek Instruments Inc., Winooski, VT, USA). Gen 5 software (BioTek Instruments Inc.) generated antibody responses from the standard curve.

2.6 | Cytokine responses to schistosomula antigens

Stored peripheral blood mononuclear cells were thawed, stimulated with rSmKK7, rSLy6A, rSmLy6B and rSmTSP7 and the supernatant collected after 24 h and tested for cytokines, chemokines and growth factors as described in the companion paper (Egesa unpublished).

2.7 | Statistical methods

Antibody (IgG1, IgG4 and IgE) levels against the schistosomula antigens before treatment were categorized as antibody responders and nonresponders. Antibody responders were participants with antibody levels greater than the mean plus 3 standard deviations against the schistosomula antigens detected in the plasma of 26 nonendemic European individuals. Factors associated with detectable pre-treatment or post-treatment antibody (IgG1, IgG4 and IgE) responses against crude schistosome and schistosomula antigens were determined using multiple logistic regression. The correlation between the antibody levels and cytokine responses to schistosomula antigens among antibody responders was investigated using Spearman's rank correlation. Multivariable logistic regression was used to investigate whether detectable five weeks post-treatment antibody responses were associated with one year post-treatment reinfection intensity. Because antibody levels were not normally distributed, the levels were log-transformed and the transformed antibody levels compared before and 5 weeks after treatment among responders, using the paired *t*-test. Statistical analysis was performed using Stata version 13 (Stata Corp, College Station, TX, USA) and graphs drawn using GraphPad Prism version 6.0 g (GraphPad Software, Inc., , San Diego, CA, USA).

3 | RESULTS

3.1 | Demographics of the study participants

Table 1 shows the demographics of the Namoni study participants at baseline. The age range of the study participants was 6-40 years. There were slightly more females than males. The study participants mainly had a heavy infection intensity (>400 epg) before treatment.

The majority of the study participants spent no longer than one hour a day in the lake (Table S1). However, the male study participants spent more time in the lake than their female counterparts (X^2 (5, N = 226) = 20.24, P = 0.001). There was no age difference in water contact behaviour (X^2 (10, N = 226) = 16.37, P = 0.089).

3.2 | Number of antibody responders to the schistosomula antigens

The number and percentage of responders are shown in Table 2. There was a substantial number of IgG1 responders to the schistosomula antigens rSmKK7 (63.3%), rSmLy6b (76.3%) and rSmTSP7 (43.8). Generally, the prevalence of IgG4 responders to the schistosomula antigens was below 22%. IgE responders to rSmKK7 (26.3%) and SmLy6b (43.3%) were observed among the study participants. The number of IgG1, IgG4 and IgE responders to the crude *S. mansoni* AWA and SEA was relatively high compared to the schistosomula antigens.

3.3 | Age and sex were associated with pre-treatment and post-treatment antibody responses against the crude antigens among antibody responders

Tables 3 and S2 show factors associated with pre-treatment and five weeks post-treatment antibody responses against crude *S. mansoni* antigens, respectively.

TABLE 2 Antibody responders to the schistosomula antigens

	Antibody responders, n (%)[a]					
Antibody	SmKK7	SmLy6a	SmLy6b	SmTSP7	AWA	SEA
IgG1	152 (63.3)	32 (13.33)	182 (76.3)	105 (43.8)	220 (91.7)	223 (92.9)
IgG4	51 (21.3)	9 (3.8)	28 (11.7)	15 (6.3)	219 (91.3)	233 (97.1)
IgE	63 (26.3)	9 (3.8)	104 (43.3)	13 (5.4)	123 (51.3)	75 (31.3)

[a]As a percentage of the 240 individuals whose data were analysed.

Before treatment, being male was associated with higher pre-treatment IgG1 to AWA ($P = 0.013$) and IgE to AWA ($P = 0.005$) and SEA ($P = 0.029$) after adjusting for gender. Being 10 years and over was associated with higher IgE levels against AWA ($P = 0.031$).

Table S2 shows factors that were associated with five weeks post-treatment antibody responses against the crude *S. mansoni* antigens, respectively. Five weeks following treatment, being male was associated with higher IgE to AWA ($P = 0.015$) after adjusting for age. Being 10 years and over was associated with higher five weeks post-treatment IgE levels against AWA after adjusting for gender ($P = 0.025$).

3.4 | Being male was associated with pre-treatment and post-treatment antibody responses against the recombinant schistosomula antigens among antibody responders

Tables 4 and S3 show factors that were associated with pre-treatment and five weeks post-treatment antibody responses against the recombinant schistosomula antigens among responders, respectively. Before treatment, being male was associated with higher IgG1 to rSmKK7 ($P = 0.009$) and rSmLy6a ($P = 0.006$). Five weeks following treatment, being male was associated with higher IgG4 levels against SmKK7 ($P = 0.0001$) and SmLy6B ($P = 0.045$).

3.5 | Those reinfected after treatment had high pre-treatment intensity

Predisposition describes the phenomenon where those that are re-infected after treatment tend to be the same individuals who had high pre-treatment intensity. The pre- and one year post-treatment infection intensity in these individuals was positively correlated ($r^2 = 0.418$, $P < 0.0001$) (Figure 2). Having a moderate pre-treatment intensity decreases the log odds of being resistant to reinfection by 1.4 ($P = 0.014$), compared to having a light infection intensity (Data not shown). In addition, having a heavy pre-treatment intensity decreased the log odds of being resistant to reinfection by 1.3 ($P = 0.010$), compared to those with a light infection intensity. Those resistant to reinfection were defined as having no eggs one year after treatment. Overall, the effect of pre-treatment intensity on re-infection intensity (and subsequently resistance to reinfection) was statistically significant after adjusting for age and gender ($P = 0.011$).

3.6 | Five weeks post-treatment antibody responses against schistosomula antigens were not associated with one year post-treatment reinfection intensity among antibody responders

To assess whether antibody responses to schistosomula antigens predicted reinfection, the association between detectable five

TABLE 3 Factors associated with pre-treatment IgG1, IgG4 and IgE detectable response against crude *Schistosoma mansoni* antigens

			IgG1		IgG4		IgE	
Antigen	Factor	Level	Adjusted[a] OR (95% CI)	*P*-value	Adjusted[a] OR (95% CI)	*P*-value	Adjusted[a] OR (95% CI)	*P*-value
AWA	Sex	Female	1	0.013	1	0.125	1	0.005
		Male	5.00 (1.40-17.86)		2.20 (0.80-5.99)		2.15 (1.25-3.68)	
	Age (years)	6 to 9	1	0.089	1	0.203	1	0.031
		10 to 13	4.09 (0.78-21.37)		3.32 (0.84-13.24)		2.19 (1.12-4.29)	
		14+	0.73 (0.25-2.10)		0.54 (0.15-2.01)		2.17 (1.13-4.14)	
SEA	Sex	Female	1	0.290	1	0.368	1	0.029
		Male	1.81 (0.60-5.48)		2.17 (0.40-11.73)		1.87 (1.07-3.26)	
	Age (years)	6 to 9	1	0.037	1	0.560	1	0.854
		10 to 13	4.67 (0.51-43.19)		3.54 (0.35-35.33)		0.82 (0.41-1.65)	
		14+	0.41 (0.13-1.35)		1.41 (0.26-7.37)		0.88 (0.45-3.00)	

OR, odds ratio.
[a]Adjusted for either sex or age.

TABLE 4 Factors associated with pre-treatment IgG1, IgG4 and IgE detectable responses against *Schistosoma mansoni* schistosomula antigens

			IgG1		IgG4		IgE	
Antigen	Factor	Level	Adjusted[a] OR (95% CI)	*P*-value	Adjusted[a] OR (95% CI)	*P*-value	Adjusted[a] OR (95% CI)	*P*-value
SmKK7	Sex	Female	1	0.009	1	0.143	1	0.625
		Male	2.09 (1.20-3.65)		1.62 (0.85-3.07)		0.86 (0.48-1.56)	
	Age (years)	6 to 9	1	0.739	1	0.019	1	0.943
		10 to 13	1.31 (0.66-2.60)		1.96 (0.92-4.18)		0.88 (0.42-1.84)	
		14+	1.15 (0.60-2.21)		0.66 (0.28-1.53)		0.96 (0.60-2.21)	
SmLy6a	Sex	Female	1	0.006	1	0.073	1	0.549
		Male	3.13 (1.39-7.02)		4.37 (0.87-21.89)		1.5 (0.39-5.91)	
	Age (years)	6 to 9	1	0.441	1	0.380	1	0.716
		10 to 13	0.93 (0.37-2.28)		1.18 (0.27-5.04)		0.51 (0.09-2.85)	
		14+	1.15 (0.21-1.45)		0.25 (0.03-2.31)		0.65 (0.13-3.08)	
SmLy6b	Sex	Female	1	0.304	1	0.713	1	0.438
		Male	1.39 (0.74-2.59)		1.17 (0.51-2.67)		0.81 (0.48-1.38)	
	Age (years)	6 to 9	1	0.069	1	0.001	1	0.082
		10 to 13	2.47 (1.08-5.61)		3.60 (1.33-9.76)		1.98 (1.02-3.83)	
		14+	1.10 (0.54-2.20)		0.54 (0.15-2.01)		1.12 (0.59-2.12)	
SmTSP7	Sex	Female	1	0.798	1	0.117	1	0.186
		Male	1.07 (0.63-1.81)		2.46 (0.79-7.56)		2.19 (0.68-7.00)	
	Age (years)	6 to 9	1	0.309	1	0.224	1	0.969
		10 to 13	1.58 (0.82-3.06)		0.93 (0.29-2.96)		1.08 (0.26-4.58)	
		14+	1.54 (0.82-2.92)		0.25 (0.05-1.28)		1.19 (0.30-4.71)	

OR, odds ratio.
[a]Adjusted for either sex or age.

weeks post-treatment antibody responses against schistosomula antigens and one year post-treatment reinfection intensity was investigated. There was no consistent association between the detectable five weeks post-treatment antibody responses against schistosomula antigens and reinfection intensity one year after PZQ treatment (Table 5).

3.7 | PZQ treatment differentially affects antibody responses against the recombinant schistosomula antigens among antibody responders

The antibody levels against the recombinant schistosomula antigens pre- and post-treatment among antibody responders are shown in Figure 3. Among the recombinant antigens, IgG1 to rSmLy6A increased five weeks post-treatment ($P < 0.0001$). On the other hand, levels of IgG1 to rSmKK7 ($P < 0.0001$), rSmLy6B ($P < 0.0001$) and rSmTSP7 ($P = 0.0006$) and IgE to rSmKK7 ($P < 0.0001$) and rSmLy6B ($P < 0.0001$) dropped five weeks following treatment. IgG4 levels to the schistosomula antigens were not affected by the treatment, with the exception of IgG4 to rSmTSP7 ($P = 0.0025$). The lack of an IgG4 response is likely due to the fact that there were very few IgG4 responders to the recombinant schistosomula antigens.

The effect of PZQ treatment on crude antigens AWA and SEA among antibody responders is shown in Figure S1. IgG1 to the crude antigens AWA ($P < 0.0001$) and SEA ($P < 0.0001$), IgG4 to AWA ($P < 0.0001$) and IgE to AWA ($P = 0.003$) increased five weeks post-treatment. IgG4 ($P = 0.526$) and IgE ($P = 0.438$) levels to SEA were not affected by treatment.

4 | DISCUSSION

Schistosomula are vulnerable to both complement and antibody-dependent cell-mediated cytotoxicity in in vitro experiments.[9-11,41,42] This makes the newly transformed schistosomulum a plausible source of candidate antigens for a vaccine against *S. mansoni*.[43] There is information on antibody responses to various *Schistosoma* antigens, but protective antibody responses against schistosomula have not been identified yet and factors that affect these antibody responses are unknown. In this study, we have presented an analysis of antibody responses to schistosomula-enriched antigens in a cohort from an endemic area. Not surprisingly, we found that being male was associated with high pre- and five weeks post-treatment antibody responses against the schistosomula antigens SmKK7 and

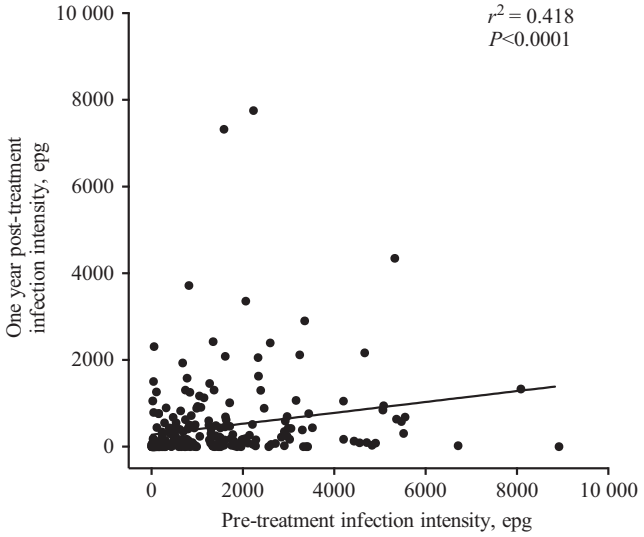

FIGURE 2 The correlation between the pre-treatment and one year post-treatment infection intensity of the study participants (n = 240)

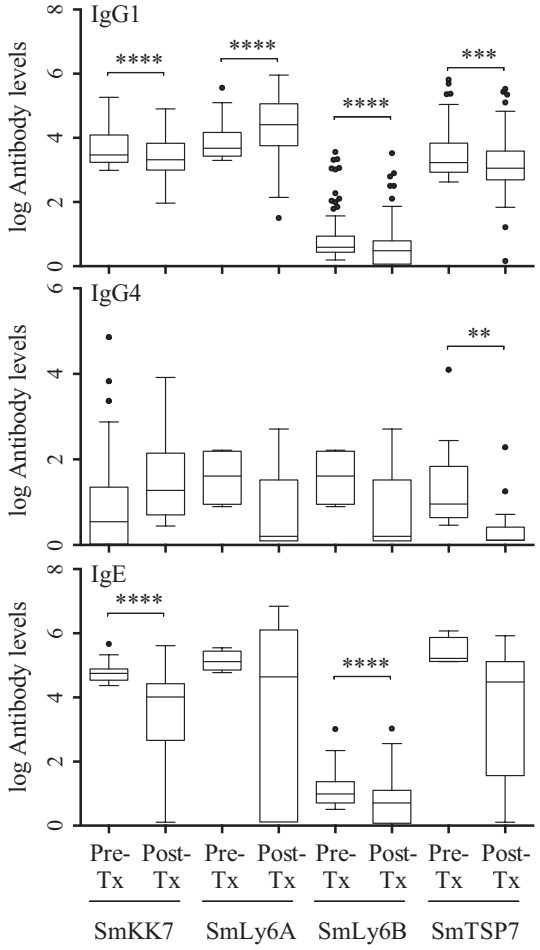

FIGURE 3 The effect of treatment on antibody responses against the recombinant schistosomula antigens SmKK7 (IgG1: n = 135, IgG4: n = 20 and IgE: n = 51), SmLy6A (IgG1: n = 25, IgG4: n = 3 and IgE: n = 4), SmLy6B (IgG1: n = 163, IgG4: n = 3 and IgE: n = 90) and SmTSP7 (IgG1: n = 90, IgG4: n = 8 and IgE: n = 8) among antibody responders. The boxes indicate the interquartile range with median as the horizontal line, while the whiskers indicate minimum and maximum antibody levels. The dots are outliers. Pre-Tx, pre-treatment antibody levels; Post-Tx, 5 weeks post-treatment antibody levels; * $P < 0.0125$ (P-value adjusted to take into account the multiple comparison), ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

rSmLy6a. The sex bias in antibody responses is likely due to the males in the present study spending more time in the lake per day than females, linked to their differing occupations, as has been reported previously.[44] Although age influences immune responses to *Schistosoma* adult worm antigens,[45,46] the antibody responses against recombinant schistosomula antigens used in this study were not age dependent. This could imply that age does not influence humoral responses to the early stages of schistosome development. This age-independent phenomenon may be attributed to the transient nature of the post-penetration schistosomula in the host skin.[47] The host is briefly exposed to the schistosomula in a natural situation, and therefore, the required antigen threshold is probably not reached to build immunity.[48,49] This may suggest that cumulative exposure that is closely related to age of the host may have no impact on immune responses to schistosomula antigens. The implication this has on vaccine design is that vaccines based on schistosomula antigens could provide prolonged exposure to a sufficient amount of schistosomula antigen and generate the same level of protection irrespective of age of the host. On the other hand, the present study observed that sex and age were associated with specific antibody responses to schistosome crude antigens AWA and SEA consistent with previous data.[45] Where participants had both antibody and cytokine data described in the companion paper (n = 54), very few weak correlations between the antibody and cytokine responses were significant.

Antibody responses to *Schistosoma* antigens are affected by treatment with PZQ.[20,50] Praziquantel (PZQ) is the most effective drug for treatment of schistosomiasis [51] and is thought to disrupt the regulation of calcium (Ca^{2+}) ion permeability through surface membranes.[52] The resulting Ca^{2+}-induced contractions are sustained, which simultaneously paralyse the parasite and dislodge them from venules. PZQ also damages the worm's body surface by forming fragile blebs and vacuoles in the schistosome tegument.[53] The blebs and vacuoles rupture, damaging the surface and facilitating host immune responses against schistosomes.[54,55] In addition, treatment exposes sequestered antigens and antibody responses towards these exposed antigens are elevated.[54,55] Although the effect of PZQ on the schistosomula is thought to be limited,[56] treating schistosomula in vitro with drugs (PZQ and oxamniquine) exposes a magnitude of sequestered antigens that are otherwise not accessible on living intact schistosomula.[15] However, not all *Schistosoma* antigens induce an increase in antibodies following PZQ treatment. For example, IgG1 levels against rSmLy6A and rSmLy6B have been shown to drop following PZQ treatment among antibody responders.[16] Similarly, we also found a drop in IgG1, IgG4 and IgE to rSmKK7, rSmLy6B and rSmTSP7, respectively, after PZQ treatment among antibody responders. On the other hand, we did observe an increase to SmLy6A. These data are in apparent contradiction to a previously

TABLE 5 Association between detectable five weeks post-treatment IgG1, IgG4 and IgE responses against schistosomula antigens and reinfection intensity

Antibody	Antigen	Crude OR (95% CI)	*P*-value	Adjusted[a] OR (95% CI)	*P*-value
IgG1	rSmKK7	0.71 (0.34-1.51)	0.377	1.01 (0.44-2.30)	0.986
	rSmLy6A	0.27 (0.11-0.64)	0.004	0.43 (0.16-1.11)	0.082
	rSmLy6B	0.50 (0.24-1.07)	0.073	0.80 (0.35-1.82)	0.592
	rSmTSP7	0.93 (0.43-1.96)	0.838	1.02 (0.45-2.31)	0.956
	AWA	0.21 (0.01-3.58)	0.287	0.62 (0.04-10.47)	0.741
	SEA	1.59 (0.18-13.39)	0.668	2.18 (0.23-19.97)	0.489
IgG4	rSmKK7	0.49 (0.13-1.73)	0.269	0.58 (0.15-2.27)	0.439
	rSmLy6A	0.72 (0.19-2.60)	0.616	0.70 (0.18-2.76)	0.608
	rSmLy6B	0.37 (0.08-1.65)	0.193	0.42 (0.09-2.05)	0.285
	rSmTSP7	1.56 (0.46-5.16)	0.470	2.17 (0.56-8.48)	0.265
	AWA	0.89 (0.18-4.39)	0.885	1.04 (0.19-5.74)	0.964
	SEA	-	-	-	-
IgE	rSmKK7	0.74 (0.29-1.94)	0.546	0.82 (0.29-2.29)	0.701
	rSmLy6A	0.72 (0.19-2.59)	0.616	0.54 (0.14-2.09)	0.371
	rSmLy6B	0.98 (0.45-2.10)	0.950	1.34 (0.57-3.12)	0.496
	rSmTSP7	0.68 (0.18-2.43)	0.551	0.48 (0.12-1.82)	0.279
	AWA	1.29 (0.59-2.84)	0.527	1.10 (0.46-2.62)	0.825
	SEA	1.31 (0.63-2.78)	0.468	1.19 (0.53-2.71)	0.671

OR, odds ratio.
[a]Adjusted for sex and gender.

published study showing that antibodies to SmLy6A decreased after treatment.[16] Similar to the study by Chalmers et al,[16] the effect of treatment was examined in participants with an antibody response above the cut-off set using nonendemic European donors at baseline. However, the study sites differed between the two studies, and it is plausible that participants from different endemic areas within a country mount differing antibody responses to the same recombinant antigens. Our findings suggest that there is heterogeneity in the response to *Schistosoma* antigens following treatment and that some antigens induce an immune response and give rise to increased antibodies, while other antigens do not.

As *Schistosoma* has more than one life cycle stage in the definitive host, some *Schistosoma* antigens are shared across these stages of *Schistosoma* including the schistosomulum, the adult and the egg.[57] As a result, there is potential for cross-reactive antibody responses to the adult worm, the egg and the schistosomula. The schistosomula antigens tested in the present study SmKK7, SmLy6a, SmLy6b and Smtsp7 are also expressed by the adults.[14,17,57] These studies have shown that there is a low expression of the mRNA transcripts for these antigens in miracidia, sporocysts and cercariae, increasing in schistosomula and adult worms. Most importantly, the egg that induces the immunopathology associated with chronic schistosomiasis had minimal levels of transcripts. Because treatment with PZQ alters immune responses to adult *Schistosoma* antigens,[58] it could indirectly modify host immune responses to the schistosomula.[59] This implies that cross-reactive antibodies against larvae could be boosted by killing the adults and releasing antigens. Therefore, shared epitopes could be good antigens to study further for vaccines that target both the schistosomula and the adults.

In longitudinal studies, antibody responses to specific schistosome antigens have been shown to correlate with resistance to reinfection.[60,61] Generally, the antibody responses associated with human resistance to reinfection are directed against the schistosome adult worm and not egg antigens.[62] This finding may not be surprising as the immune responses to crude adult worm and egg antigens have been widely studied. However, limited work has shown that IgG [32] and IgE [31] antibody responses to larval antigens are associated with resistance to reinfection. By looking at those infected individuals who were cured five weeks following treatment, we related their reinfection intensity to antibody responses to schistosomula antigens pre-treatment and five weeks post-treatment. Determinants of reinfection (reviewed in[38]) we considered in this analysis were age and sex. In the present study, pre-treatment or five weeks post-treatment antibody responses against schistosomula antigens were not associated with one year post-treatment reinfection intensity. These findings imply that although the schistosomula antigens tested in the present study were targeted by IgG1 and IgE, the responses were not protective. With the schistosomula as the target of immune attrition, using the radiation-attenuated cercariae the only experimental vaccine to date to consistently provide high levels of protective immunity (60%-80%) against challenge,[63-65] there is a need to identify other schistosomula antigens that are targets of protective immunity in humans. It could be that other, perhaps non-protein, schistosomula molecules such as glycans may be targets of protective immunity in humans. A glycomic analysis of the life cycle of *S. mansoni* shows stage-specific expression of glycans, including during the schistosomula stage.[66] Glycans are highly antigenic,[67] and schistosomula glycans are the dominant targets of antibody

responses.[68-70] There is evidence that schistosome glycans induce protective immune responses. For instance, anti-glycan IgG protects mice against challenge with *S. japonicum*.[71] Furthermore, glycan-specific IgG antibodies have been associated with the ability of non-human primates to naturally clear *Schistosoma* infections.[72] In the present study, the recombinant schistosomula proteins were generated in an *E. coli* system without the ability to glycosylate proteins. Therefore, the antibody responses to schistosomula glycoproteins could not be established. As it is now possible to use an engineered *E. coli* system to produce recombinant glycoproteins,[73,74] schistosomula glycans (in the form of recombinant glycoproteins) should be investigated further for a possible role in human anti-schistosome immunity and subsequently their potential as vaccine candidates.[75]

In addition to identifying the antibodies that target schistosomula antigens, another important issue to consider during the development of a schistosomiasis vaccine is whether the schistosomula antigens induce IgE. IgE responses to worm antigens correlate with age-dependent human immunity,[20,76-78] but IgE has also been observed to mediate allergic reactions to helminth antigens in previously infected people.[23] In the present study, the relatively high IgE responses to rSmKK7 and rSmLy6B would indicate that these antigens could be potentially allergenic if developed further as vaccine candidates and thus are probably not a good avenue for further development. This finding could point to a new class of possible IgE-binding protein families that have never been seen before. Whether the IgE levels to the schistosomula antigens are sufficient to lead to allergic responses/cross-reactivity remains to be investigated. Possible vaccine-induced allergic reactions can be mitigated when selecting vaccine antigens by in silico screening out of potentially allergenic antigens using bioinformatics tools, such as the Structural Database of Allergenic Proteins (SDAP), that predict antigens that IgE binds.[79] This was, in fact, performed for the schistosomula antigens used in this study, by TheSchistoVac (http://www.theschistovac.eu/). Our present study cohort (the Namoni cohort) is a similar cohort to the Musoli cohort (also in Uganda) but showed that there were individuals with high IgE responses to some of the schistosomula antigens. There appears to be heterogeneity in the response to schistosomula, even between geographically close populations (Musoli vs Namoni). It may be that across a population, there will be people that respond to any novel *Schistosoma* antigen with IgE, and it may not be possible to screen this reactivity out, meaning that there may be some people who will end up with an adverse reaction to any novel antigen. Of course, if the risk is small and can be managed, then the benefit of administering an antischistosome vaccine will far outweigh the risks.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DISCLOSURES

None.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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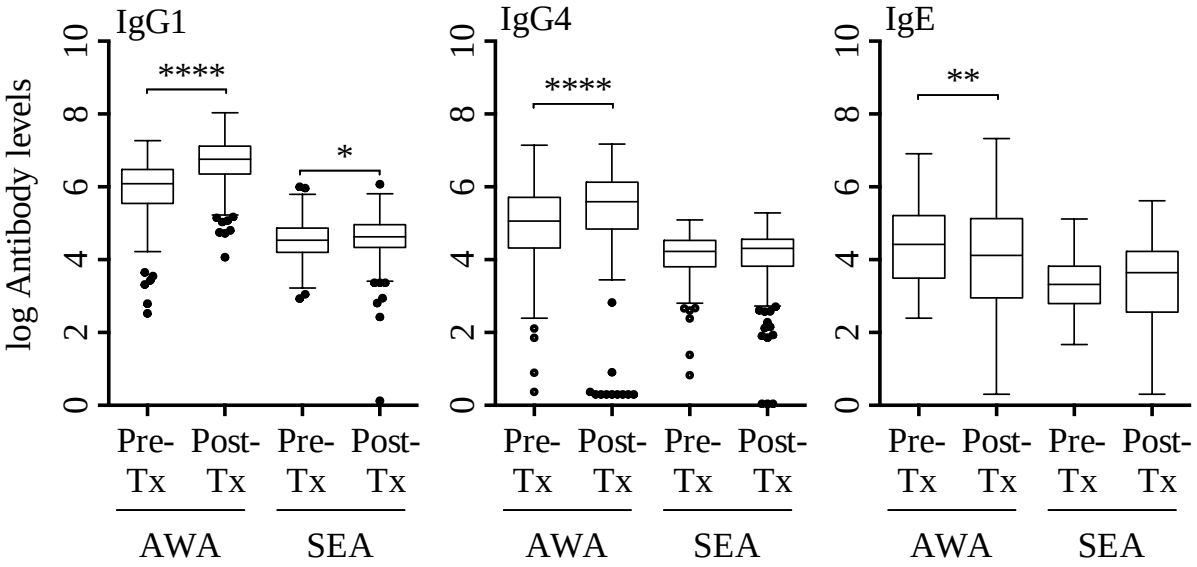

Figure S1 The effect of treatment on the antibody responses against the *S. mansoni* crude antigens AWA (IgG1; n=201, IgG4; n=200 and IgE; n=109) and SEA (IgG1; n=203, IgG4; n=211 and IgE; n=67) among antibody responders. The boxes indicate the interquartile range with median as the horizontal line while the whiskers indicate minimum and maximum antibody levels. The dots are outliers. Pre-Tx, Pretreatment antibody levels; Post-Tx, 5-week post-treatment antibody levels; *p<0.025 (p-value adjusted to take into account the multiple comparison), **p<0.01, ***p<0.001, ****p<0.0001

1 **Supplementary Tables**

2 **Table S1.** Water Contact Details of the Study Participants (N=226)

3

Water contact activity	**Level**	**n (%)**
Spending time in lake (hours/day)	<0.5	83 (36.7)
	0.5-1	108 (47.8)
	1-2	13 (5.7)
	2-3	6 (2.7)
	3-4	5 (2.2)
	>4	11 (4.9)
Fishing in the lake	No	201 (88.9)
	Yes	25 (11.1)
Washing or processing fish in lake	No	188 (83.2)
	Yes	38 (16.8)
Washing clothes or utensils in lake	Yes	176 (77.9)
	No	50 (22.1)
Swimming / bathing / playing in the lake	No	164 (72.6)
	Yes	62 (27.4)
Fetching water from the lake	No	206 (91.2)

	Yes	20 (8.8)
Going to the lake for transport	No	174 (77.0)
	Yes	52 (23.0)
Going to the lake for farm irrigation	No	183 (81.0)
	Yes	43 (19.0)
Going to the lake for anything else	No	119 (52.7)
	Yes	107 (47.3)

4

5

6 **Table S2.** Factors Associated with Five-Week Post-Treatment IgG1, IgG4 and IgE Levels Against Crude Antigens

7

8

Antigen	Factor	Level	IgG1		IgG4		IgE	
			Adjusted† OR‡ (95% CI)	P–value	Adjusted† OR‡ (95% CI)	P–value	Adjusted† OR‡ (95% CI)	P–value
AWA	Sex	Female	1		1	0.631	1	0.015
		Male	-	-	1.33 (0.42–4.27)		2.01 (1.15–3.55)	
	Age (years)	6 to 9	1	0.625	1	0.722	1	0.025
		10 to 13	4.09 (0.78–21.38)		3.32 (0.09–13.23)		2.19 (1.12–4.28)	
		14+	0.73 (0.25–2.10)		1.11 (0.41–3.05)		2.17 (1.13–4.15)	
SEA	Sex	Female	1	0.372	1	0.754	1	0.128
		Male	1.54 (0.60–1.71)		0.73 (0.10–5.38)		1.50 (0.89–2.53)	
	Age (years)	6 to 9	1	0.185	1	0.861	1	0.379
		10 to 13	468 (0.51–43.20)		3.54 (0.35–35.33)		0.82 (0.41–1.65)	
		14+	0.41 (0.13–1.35)		1.41 (0.27–1.7.37)		0.89 (0.45–1.74)	

† OR Odds Rati , ‡ Adjusted for either sex age

9 **Table S3.** Factors Associated with Five-Week Post-Treatment IgG1, IgG4 and IgE Levels Against Schistosomula Antigens

Antigen	Factor	Level	IgG1		IgG4		IgE	
			Adjusted† OR‡ (95% CI)	P–value	Adjusted† OR‡ (95% CI)	P–value	Adjusted† OR‡ (95% CI)	P–value
SmKK7	Sex	Female	1	0.125	1	0.001	1	0.308
		Male	1.52 (0.89–2.59)		3.49 (1.63–7.44)		1.37 (0.74–2.50)	
	Age (years)	6 to 9	1	0.070	1	0.254	1	0.528
		10 to 13	1.48 (0.75–2.92)		1.96 (0.91–4.18)		0.88 (0.42–1.84)	
		14+	0.70 (0.37–1.32)		0.66 (0.29–1.53)		0.96 (0.47–1.93)	
SmLy6a	Sex	Female	1	0.038	1	0.120	1	0.146
		Male	1.76 (1.03–3.02)		1.90 (0.85–4.25)		1.84 (0.81–4.18)	
	Age (years)	6 to 9	1	0.002	1	0.822	1	0.391
		10 to 13	1.51 (0.78–2.90)		1.38 (0.51–3.74)		0.51 (0.09–2.86)	
		14+	0.47 (0.24–0.90)		1.16 (0.43–3.16)		0.65 (0.14–3.08)	
SmLy6b	Sex	Female	1	0.718	1	0.602	1	0.421
		Male	1.11 (0.64–1.90)		1.22 (0.57–2.62)		1.24 (0.72–2.13)	
	Age (years)	6 to 9	1	0.0062	1	0.135	1	0.149
		10 to 13	2.47 (1.08–5.61)		3.60 (1.33–9.76)		1.98 (1.03–3.83)	
		14+	1.10 (0.54–2.20)		0.54 (0.15–2.01)		1.12 (0.59–2.12)	

SmTSP7	Sex	Female	1	0.965	1	0.045	1	0.168
		Male	1.01 (0.60–1.71)		2.66 (0.85–4.25)		1.79 (0.78–4.16)	
	Age (years)	6 to 9	1	0.188	1	0.633	1	0.131
		10 to 13	1.58 (0.82–3.06)		0.93 (0.29–2.96)		1.09 (0.26–4.58)	
		14+	1.54 (0.82–2.92)		0.25 (0.05–1.28)		1.19 (0.30–4.71)	
† OR Odds Rati , ‡ Adjusted for either sex age								

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