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Schistosoma haematobium infection modulates *Plasmodium falciparum* parasite density and antimalarial antibody responses

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Abstract

Aims: Schistosomiasis and malaria are endemic in sub-Saharan Africa where *Schistosoma haematobium* (*Sh*) and *Plasmodium falciparum* (*Pf*) coinfections are thus frequent. We explored the effect of *Sh* infection on antibody responses directed to *Pf* merozoite antigens and on malaria susceptibility in Beninese children.

Methods and results: A total of 268 children were followed during a malaria transmission season. Detection of *Pf* infection was performed by microscopy and rapid diagnostic tests. *Sh* infection was determined in urine by microscopy. Antimalarial antibody, cytokine and HLA-G concentrations were quantified by ELISA. The expression of HLA-G receptors by immune cells was assessed by flow cytometry. Children infected by *Sh* had higher concentrations of IgG1 directed to MSP3 and $GLURP_{R0}$, IgG2 directed to $GLURP_{R0}$ and IgG3 directed to MSP3, $GLURP_{R0}$ and $GLURP_{R2}$ and have lower *Pf* densities than those uninfected by *Sh*. No difference in cytokine and HLA-G concentrations was observed between *Sh* egg carriers and non-carriers.

Conclusion: *Schistosoma haematobium* modulates host immune responses directed to *Pf* antigens. The absence of immune downregulation usually observed during helminth infections is surprising in our study. We hypothesize that the stage of *Sh* development could partly explain the immune pathways leading to increased antibody levels that favour better control of *Pf* parasitemia.

KEYWORDS
coinfection, HLA-G, LILRB1 and LILRB2, malaria, *P falciparum*, *S haematobium*

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1 | INTRODUCTION

Schistosomiasis and malaria are common tropical parasitic diseases, and they cause a high burden of morbidity and mortality, particularly in children.[1,2] Given the overlap of the geographical distributions of *Schistosoma haematobium* (*Sh*) and *Plasmodium falciparum* (*Pf*), the risk of coinfection is frequent in sub-Saharan Africa.

Several epidemiological studies have explored the interactions between the two parasites and their consequences in terms of malaria susceptibility. However, they have generated contradictory findings. Lower parasite densities (PD) and a lower incidence of *Pf* infection or malaria-related complications in children infected by *Sh* have been reported,[3-7] whilst other studies have reported an increased risk of *Pf* infections or higher PD associated with *Sh* or *S mansoni* coinfections.[8-12] The lack of an association between *Schistosoma* infection and the risk of *Pf* infection has also been reported in Kenya, Ethiopia and Uganda.[13,14] Differences in parasite exposure and related anti-parasitic immunity, study population and design, as well as the sensitivity of diagnostic tools could partly explain these discordant observations.

Host immune responses that are able to control helminth and *Plasmodium* infections are different mainly due to the life cycle of each parasite species, and they vary according to the developmental stage of the infection.[15,16] Schistosome infections typically result in a series of immune events dominated by a T helper Type 2 (Th2) immune response, characterized by activation of eosinophils, basophils and mast cells, high levels of immunoglobulin E (IgE) and proliferation of T regulatory cells during the chronic stages of the disease. *Pf* infection rapidly triggers a Th1-type immune response, associated with high concentrations of IFN-γ and TNF-α that are essential for the control of asexual blood stage parasite densities. The Th1-type immune response is followed by a Th2 immune response, associated with production of cytophilic immunoglobulin G antibodies, that interfere with the development of asexual blood stages and that can act in cooperation with innate immune cells. The host immune system thus seems to face a dilemma in terms of reacting efficiently to the two parasites when coinfected.[17]

Studies concerning the impact of schistosomiasis on anti-*Pf* antibody (Ab) responses are limited. Five studies have analysed anti-*Pf* Ab responses during schistosome infection.[18-22] Three of them documented lower levels of IgG with specificity for *Pf* candidate vaccine antigens or crude schizont extracts in *Sh*-infected individuals.[18,19,21] Conversely, another of them found a higher concentration of schizont extract- and $MSP1_{-19}$-specific cytophilic IgG in the *Pf*/*Sh* coinfection group.[20] Finally, a study in Zimbabwe reported no difference in levels of antibodies either with specificity for crude *Pf* antigens or with parasite growth inhibitory activity between different groups.[22] Here again, differences of exposure, study population and study design could explain these discordant findings.

Negative associations between *Sh* infection and the levels of IgG directed to plasmodial antigens could be rather associated with chronic schistosome infection and the related immunomodulatory mechanisms that ensure helminth persistence within the host, whilst more recent, comparatively less chronic *Sh* infection could be associated with higher Ab levels in the absence of well-established immune-regulatory mechanisms. The IL-10 is known to be strongly involved in these regulatory mechanisms, but other molecules such as human leucocyte antigen G (HLA-G) could also participate in establishing an immunoregulatory state. HLA-G is known to inhibit the function of a panel of immune cells, including B cells, through direct interaction with its receptors LILRB1 and LILRB2.[23,24] Moreover, HLA-G is associated with susceptibility to other parasitic diseases such as malaria and human African trypanosomiasis.[25-29] We hypothesize that induction of HLA-G expression could contribute to the control of host immune responses during helminth infections.

Our objectives were to assess the effect of *Sh* infection on the Ab concentrations directed to *Pf* merozoite antigens and on the susceptibility to malaria in Beninese children. Moreover, we determined the association between *Sh* and *Pf* coinfections with the production of plasmatic cytokines and soluble HLA-G and the expression level of LILRB1 and LILRB2 inhibitory receptor on immune cell subsets.

2 | MATERIAL AND METHODS

2.1 | Study site and population

The study was conducted in the Sô-Ava district, southern Benin where both parasites are endemic.[30,31] The study population comprises predominantly the Toffin ethnic group. They mostly live in stilt houses built on lake Nokoué and make their living primarily through fishing and, to a smaller extent, trading and farming on the banks of the lake.[32]

2.2 | Study design

Active and passive parasitological and clinical surveillance for malaria was conducted in a total of 268 school-aged children recruited between May and July 2013, beginning of the heavy malaria transmission season. Parasitological measurements consisted of repeated monthly examination using thick blood smears over a 6-month surveillance period. In case of fever, both a rapid diagnostic test (RDT) and a thick blood smear (TBS) were carried out. Symptomatic malaria infection (fever or history of fever in the preceding 24 hours and positive TBS and/or RDT) was treated according to the recommendations of the national malaria control programme. Moreover; parents were invited to bring their children to the private St Joseph Medical health centre at any time in case of suspicion of fever or clinical signs, whether related to malaria or not. Children with schistosome eggs in urine samples (for details, see below) received curative treatment with praziquantel (40 mg/kg). Medical consultations and treatments were free of charge for all the participants. For all children, individual characteristics were recorded (Table 1). The study was explained in detail to all participants' parents or guardians, who gave their signed informed consent. The study was approved by the

Ethics Committee of the Faculté des Sciences de la Santé, Cotonou, Benin and registered at No.12/03/2012/CEIFSS/UAC.

2.3 | Detection of *Pf* and helminth infections

Malaria infection was diagnosed in children through TBS and/or RDT. TBS were prepared systematically during cross-sectional surveys and when fever was detected during medical consultations and were stained with 10% Giemsa prior to microscopy. TBS allowed for quantification of PD and identification of asymptomatic infections, with results reported as the number of infected red blood cells/8000 leucocytes. During medical visits, RDT were systematically performed when fever was detected in order to provide a rapid diagnosis. *Sh* infection was diagnosed by microscopy to detect *Sh* eggs in urine. Urine samples were first homogenized to ensure even dispersal of eggs before examination. Kato-Katz faecal smears were performed to detect soil-transmitted helminth infections.

2.4 | Antibody, cytokine and HLA-G measurements

The quantification of IgG1, IgG2 and IgG3 antibodies directed to $GLURP_{R0}$ (amino acids 25-514), $GLURP_{R2}$ (amino acids 706-1178) and MSP3 (amino acids 212-380) was performed by ELISA following the protocol described elsewhere.[33] GLURP and MSP3 antigens were selected because IgG levels directed to these antigens were previously associated with malaria protection in different cohorts.[34-37] Concentrations of IL-4, IL-10, TNF-α and IFN-γ were determined using BD OptEIA ELISA Set (BD Biosciences) following the manufacturer's protocol and previously described by our team.[19] The levels of soluble HLA-G isoforms HLA-G1 and HLA-G5 were determined by ELISA assay as described by elsewhere.[27] Details on the ELISA procedures used for antibody, cytokine and HLA-G quantifications are presented in the Table S1.

2.5 | Cellular phenotyping and HLA-G receptor expression

Blood samples (1 mL) from a subgroup of 50 participants (25 *Sh*- and 25 *Sh*+) were subjected to phenotype analysis. The expression of LILRB1 and/or LILRB2 inhibitory receptors on the surface of the following cell populations was analysed: T helper cells ($CD3^+CD4^+$), CD8 T cells ($CD3^+CD8^+$), T regulatory cells ($CD3^+CD4^+CD25^{high}CD127^-$), T effector cells ($CD3^+CD4^+CD25^+CD127^+$), γδ T cells ($CD3^+Vd2^+$) and B cells ($CD19^+$). Monocytes were characterized by expression of $CD14^+$ in the population of mononuclear cells. Neutrophils and eosinophils are respectively $CD16^+$ and $CD16^-$ in the polymorphonuclear cell population. To define positive and negative events, isotype-matched control antibodies were used. The gating strategy used to distinguish the different cell populations is presented in Figure 1. FlowJo software was used for the flow cytometric data analysis.

TABLE 1 Characteristics of the study population

Characteristics of the population (N = 268)	
Age (mean in years [range])	7 [4-8]
Sex (% of male)	66 (178/268)
Ethnic group	Toffin
MLHC [IC 95%]	9.86 [9.70-10.02]
Anaemia[a] (%)	89 (238/268)
Hb defects (%)	25 (68/268)
AS	75 (51/68)
AC	22 (15/68)
SC	3 (2/68)
NIM at the end of follow-up (EF) (%)	96 (255/266)
PWRA at EF (%)	71 (188/264)
Pf-infected children at EF (%)	45 (121/268)
MiRBC at EF [range]	14 717 [32-254,400]
Sh-infected children at EF (%)	34 (90/268)
Sh infection intensity[b]	
Low (%)	20 (18/90)
Medium (%)	40 (36/90)
Heavy (%)	40 (36/90)

Abbreviations: Hb, haemoglobin; MiRBC, Mean number of *Pf*-infected RBC for 8000 leukocytes; MLHC, Mean of haemoglobin concentration in g/dL; NIM, Number of individuals using mosquito net at end of follow-up; PWRA, Practice of water-related activities (fishing, swimming, bathing, washing clothes and washing dishes). This variable was coded 1 if at least one of these activities was practised.

[a]Here, we considered the children with moderate anaemia using WHO thresholds (24) that is children 4 y old with haemoglobin level lower than 9.9 g/dL and children from 5 to 11 y old with haemoglobin level lower than 10.9 g/dL.

[b]*Sh* infection intensity was stratified in three groups: low (≤10 eggs/10 mL of urine), medium (11-50 eggs/10 mL of urine) and heavy intensity (>50 eggs/10 mL of urine). The median and range in the low, medium and heavy *Sh* infection intensity groups were respectively 10 [5-10]; 30 [15-50] and 105 [55-1165].

2.6 | Analysis strategy

To compare our results with those published in Courtin et al, 2011,[19] we focused our analysis on the classification of the *Sh*-negative and *Sh*-positive groups performed at the end of the follow-up. First, the antibody concentrations, the prevalence of *Pf* infection and the mean number of *Pf*-infected RBC detected at the end of the follow-up were compared in the *Sh*-negative and *Sh*-positive groups by univariate analysis (Mann-Whitney test; Student's *t* test and chi-square test). Variables with $P < .10$ were included in the multivariate models. Quantitative variables with abnormal distribution were normalized using log transformation. Linear regression multivariate models were performed to assess the influence of *Sh* infection on IgG1, IgG2 and IgG3 directed against *Pf* antigens, and PD.

Then, the mean level of *Pf* parasitemia calculated during the whole follow-up and the last trimester of follow-up were compared between the *Sh*-infected status at the end of the follow-up using

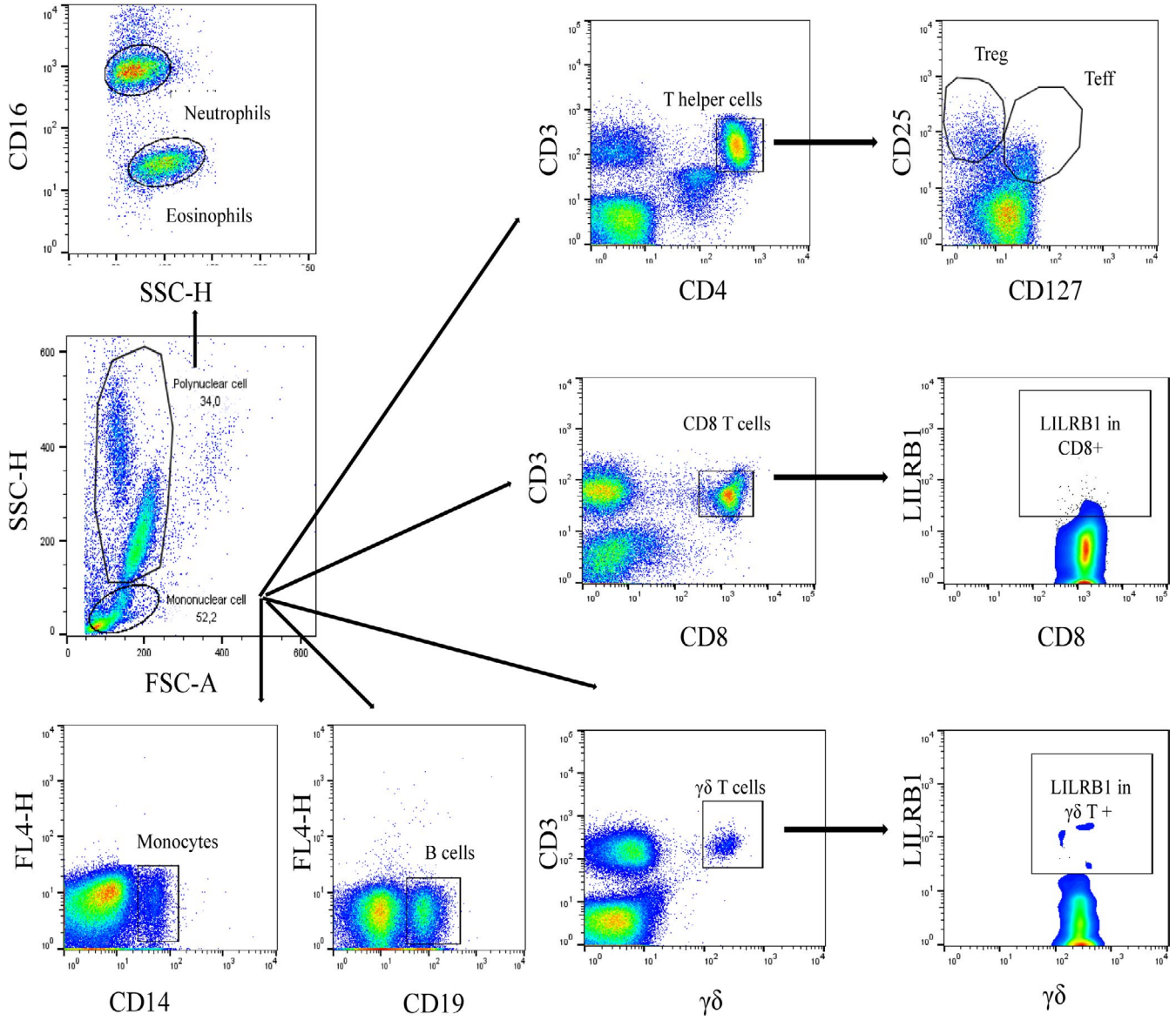

FIGURE 1 Gating strategy. Gating strategy used to distinguish (A) mononuclear and polynuclear cells based on forward scatter and side scatter, (B) Neutrophils and Eosinophils based on CD16+ (C) Th cells based on CD4 + and CD3+, (D) T regulator and T effector lymphocytes based on CD25 + and CD127+, (E) CD8 T cells based on CD3 + and CD8+, (F) LILRB1 expression in CD8 cells based on LILRB1 + and CD8+, (G) Monocytes based on CD14+, (H) B cells based on CD19+, (I) γδT cells based on CD3 + and $Vd2^+$, (J) LILRB1 expression in γδT cells based on LILRB1 + and $Vd2^+$

linear regression multivariate model. The mean level of *Pf* parasitemia was also dichotomized in qualitative variables as described in the Table 6. We used a logistic regression model with different thresholds to assess the role of *Sh* infection on modulating PD.

3 | RESULTS

3.1 | Characteristics of study population

The general characteristics of the study population (n = 268) are presented in Table 1. All the children included in the study were from the Toffin ethnic group. Their mean age was 7 years old with more boys (66%). About 89% of the children had at least moderate anaemia, and 25% of them were carriers of a haemoglobinopathy. About 45% and 34% of the children were respectively infected by *Pf* and *Sh* at the last cross-sectional medical visit.

3.2 | Malaria infections during the follow-up

Data collected during the 6-month malaria follow-up are presented in Table 2. About 89% of children had at least one *Pf* infection during follow-up. Among them, 47% had at least one asymptomatic infection and 84% had at least one symptomatic infection. During the follow-up, 676 symptomatic and 176 asymptomatic *Pf* infections were reported,

TABLE 2 Data collected during the malaria follow-up

Characteristics	Values
Frequency (Fq) of children with at least one *Pf*-infection (%)[a]	89 (235/264)
Mean number of *Pf* infections per child [range]	3 [0-8]
Total number of *Pf* infections	856
Class 1 (1 to 2 infections (%))	34 (80/235)
Class 2 (3 to 4 infections (%))	37 (87/235)
Class 3 (5 to 8 infections (%))	29 (68/235)
Fq of children with at least one symptomatic *Pf* infection (%)	84 (223/264)
Mean number of symptomatic *Pf* infection per child [range]	3 [0-8]
Total number of symptomatic *Pf* infections	676
Class 1 (1 to 2 infections (%))	41 (97/235)
Class 2 (3 to 4 infections (%))	37 (86/235)
Class 3 (5 to 8 infections (%))	17 (40/235)
Fq of children with at least one asymptomatic *Pf* infection (%)	47 (124/264)
Mean number of asymptomatic *Pf* infection per child [range]	1 [0-5]
Total number of asymptomatic *Pf* infections	176
Class 1 (1 infection (%))	35 (82/235)
Class 2 (2 to 5 infections (%))	18 (42/235)
MiRBC during follow-up [range]	19,462 [48-737,600]
MiRBC during medical consultations [range]	25,213 [16-737,600]
MiRBC during cross-sectional monthly visits [range]	2,720 [8-57,280]

Abbreviation: MiRBC, Mean number of *Pf*-infected RBC per 8000 leucocytes.

[a]89% of the children had at least one *Pf*-infection during the follow-up. A total of 856 *Pf* infections were reported in the study. Information on presence or absence of fever was missing for four infections so it was not possible to classify the infection in the group of symptomatic or asymptomatic *Pf*-infection. Four children were not considered in our analyses because the follow-up was not optimal (≤ 2 visits).

and each child had on average 3 *Pf* infections. The mean *Pf* density during follow-up was 19 462 iRBC/8000 leucocytes, including infections detected during cross-sectional visits and during medical consultations.

3.3 | *Sh* infection and individual' characteristics

No difference was observed between *Sh* status and various parameters such as age, sex, anaemia and haemoglobinopathies (Table 3).

3.4 | *Sh* infection and immunological responses

3.4.1 | Anti-Pf IgG concentration according to Sh infection

The result of univariate analyses showed that children infected by *Sh* had higher concentrations of IgG1 to MSP3 and to $GLURP_{R0}$, of IgG2 to $GLURP_{R2}$ and of IgG3 to MSP3, $GLURP_{R0}$ and $GLURP_{R2}$ than those uninfected by *Sh* (Table 3). Children coinfected by both parasites had higher IgG1, IgG2 and IgG3 concentrations to MSP3, $GLURP_{R0}$ and $GLURP_{R2}$ than those uninfected and higher IgG1 to MSP3, IgG2 to $GLURP_{R0}$ and IgG3 to MSP3, $GLURP_{R0}$ and $GLURP_{R2}$ than children only infected by *Pf* (Figure 2). Children also infected by *Pf* at the time of blood sampling used for Ab quantification and those infected by *Pf* during the follow-up presented higher IgG1, IgG2 and IgG3 concentrations to MSP3 and GLURP antigens than children uninfected by *Pf* (Table S1). The higher anti-*Pf* IgG concentrations observed in *Sh* egg carriers were confirmed in multivariate analysis after adjustment on the factors associated with a modulation of Ab responses in univariate analyses (Table S2), including *Pf* infection (Table 4).

3.4.2 | Cytokines and HLA-G levels according to Sh infection

No significant difference was found between children infected or uninfected by *Sh* with respect to sHLA-G or cytokine concentrations (Table S3). A significant difference was only observed for IFN-γ in children infected by *Pf* when compared to those uninfected (Mann-Whitney test, $P = .02$).

3.4.3 | Immune cells and LILRB1 and/or LILRB2 expression according to Sh infection

LILRB1 and/or LILRB2 expression at the surface of a panel of immune cells was investigated in a subgroup of 50 children, half of

TABLE 3 Individual, immunological, clinical and parasitological variables according to *Sh* infection

Variables	*Sh* status		*P*
Individual variables	Absence of *Sh* egg (n = 178)	Presence of *Sh* eggs (n = 90)	
Age (mean in years) [range]	7 [4; 8]	7 [4; 8]	>.10
Sex (Male/female (%))	67/33	66/34	>.10
MLHC [IC 95%]	9.82 [9.62; 10.03]	9.93 [9.66-10.20]	>.10
Number of anaemia children (%)	90 (160/178)	87 (78/90)	>.10
RBC defects in %	23 (41/178)	30 (27/90/	>.10
AS	17 (31/178)	22 (20/90)	>.10
AC	6 (10/178)	5 (5/90)	>.10
SC	0	7 (2/90)	-
MWRA	67 (119/178)	77 (69/90)	>.10
Immunological variables[a]			
IgG1-MSP3[b]	7.26 [6.86; 7.67]	8.16 [7.66; 8.65]	.009
IgG1-GLURP$_{R0}$	8.03 [7.70; 8.35]	8.77 [8.34; 9.20]	.007
IgG1-GLURP$_{R2}$	7.61 [7.23; 7.99]	8.04 [7.49; 8.59]	>.10
IgG2-MSP3	5.49 [5.17; 5.81]	5.94 [5.50; 6.37]	>.10
IgG2-GLURP$_{R0}$	5.74 [5.42; 6.06]	6.61 [6.11; 7.10]	.003
IgG2-GLURP$_{R2}$	8.99 [8.65; 9.33]	9.24 [8.80; 9.68]	>.10
IgG3-MSP3	9.27 [8.84; 9.70]	10.80 [10.18; 11.41]	<.001
IgG3-GLURP$_{R0}$	6.11 [5.76; 6.45]	6.91 [6.34; 7.49]	.013
IgG3-GLURP$_{R2}$	9.13 [8.74; 9.53]	10.35 [9.75; 10.95]	.001
Clinical and parasitological variables			
Children with *Pf* infection at EF (%)	46 (83/178)	42 (38/90)	>.10
MiRBC at EF [range]	17 431 [32; 254400]	9290 [80; 96471]	>.10
Children with at least one *Pf* infection during the follow-up (%)	90 (157/174)	87 (78/90)	>.10
Children with at least one symptomatic *Pf* infection during the follow-up (%)	85 (148/174)	83 (75/174)	>.10
Children with at least one asymptomatic *Pf* infection during the follow-up (%)	47 (82/174)	47 (42/90)	>.10
MiRBC during follow-up [range]	24 932 [114.29; 737600]	7082 [48; 104 186.05]	.002

[a]No difference in cytokine and HLA-G expression was observed between children infected or not by *Sh*

[b]Antibody concentration to each antigen was expressed in pg/ml and was log-transformed.

whom were infected by *Sh*. The characteristics of the children are detailed in Table 5. Our results showed that children coinfected by *Pf* and *Sh* had a lower frequency of γδ T cells ($P = .014$ and $P = .045$, Mann-Whitney test) than those only infected by *Pf* ($P = .014$, Mann-Whitney test) or uninfected children ($P = .045$, Mann-Whitney test; Figure 3). Of note, LILRB1 expression on the surface of γδ T cells was inversely correlated to the frequency of γδ T cells in the different groups infected by *Pf* and/or *Sh* (Figure 3). No difference was observed between the frequency of other immune cells according to the groups of interest except for T cells and eosinophils (Figures 3 and 4). Children infected by *Pf* have higher frequency of T cells than uninfected ones ($P = .05$, Mann-Whitney test). A higher frequency of eosinophils was observed in children infected by *Sh* compared with those infected by *Pf* ($P = .003$, Mann-Whitney test). Finally, LILRB2 expression was lower at the surface of neutrophils in blood samples of children coinfected than those uninfected ($P = .03$, Mann-Whitney test (Figure 4)).

3.5 | *Sh* infection and malaria phenotypes

In univariate analyses, no association was found between *Sh* egg carriers and either the prevalence of *Pf* infection or the mean number of *Pf*-infected RBC detected at the end of the follow-up

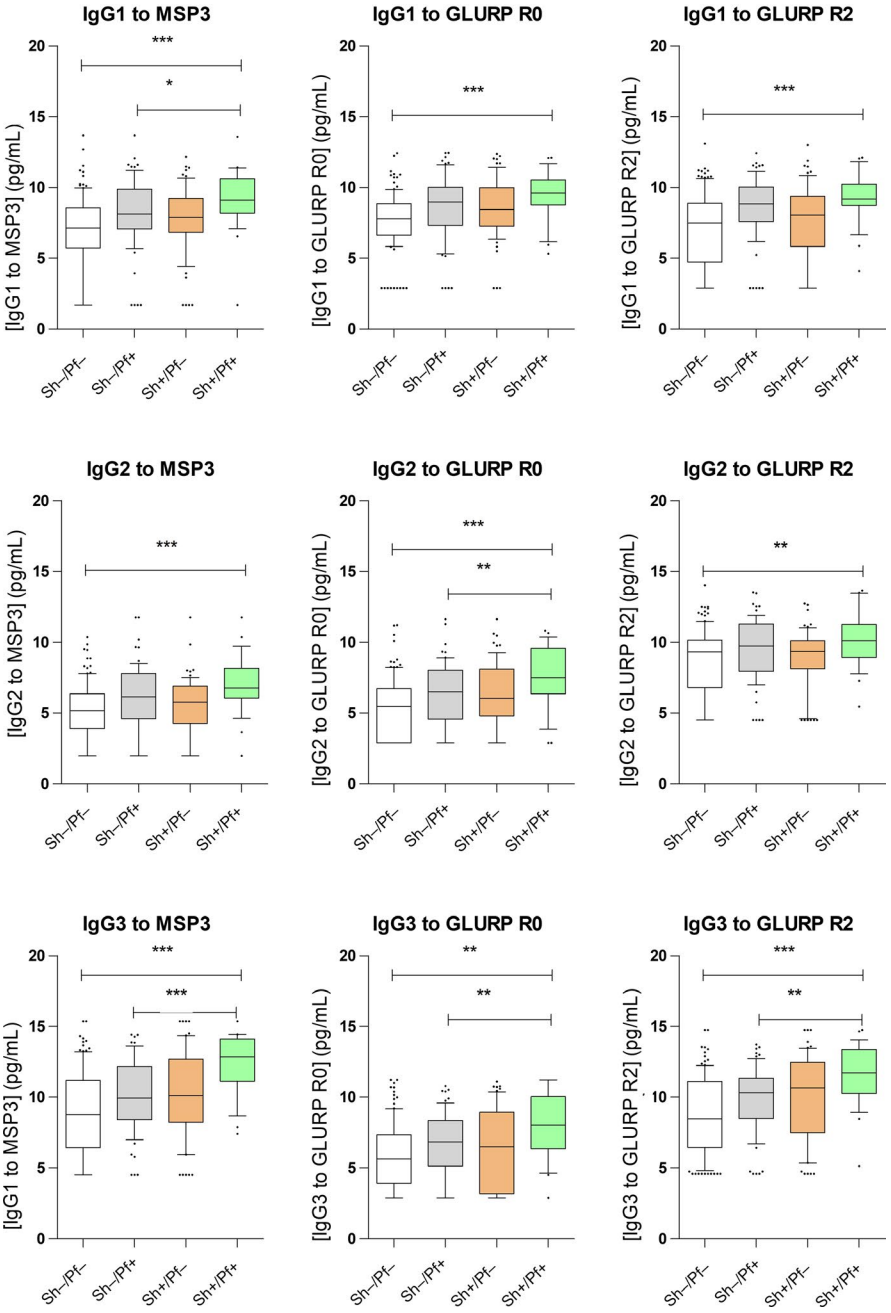

FIGURE 2 Antibody responses directed to *P falciparum* candidate vaccine antigens according to the *Sh* and *Pf* infection status. Box-whisker plots represent medians with 25th and 75th percentiles (boxes), and with 10th and 90th percentiles (whiskers). The concentrations of IgG1, IgG2 and IgG3 directed to MSP3, GLURP R0 and GLURP R2 were compared between (a) uninfected children (*Sh*-/*Pf*-; n = 95), (b) children only infected by *Pf* (*Sh*-/*Pf*+; n = 83), (c) children only infected by *Sh* (*Sh*+/*Pf*-; n = 52) and (d) children coinfected by *Sh* and *Pf* (*Sh*+/*Pf*+, n = 38) using non-parametric Mann-Whitney test. $^{*}P < .05$, $^{**}P < .01$ and $^{***}P < .001$

(Table 3). Using a retrospective approach, *Sh* egg carriers have lower mean parasitic densities of *Pf* (*P* = .002; Kruskal-Wallis test) during the follow-up than those uninfected by *Sh* (Table 3). *Sh* egg carriage did not influence the risk of having malaria infections (symptomatic or asymptomatic) during the follow-up (Table 3). Multivariate analyses were performed to explore more precisely the role played by *Sh* on *Pf* PD used as either quantitative or qualitative variables (Table 6). A clear association was observed between *Sh* infection and the mean number of iRBC/8000 leucocytes detected during the follow-up using a linear regression model after adjustment on factors influencing *Pf* PD such as sickle cell trait, age and Ab concentrations. Children infected by *Sh* had significantly lower mean PD (Coef [IC] = −0.87 [−1.41; −0.33]) than *Sh* uninfected children (Table 6). Similar trend was observed (Coef [IC] = − 0.64 [−1.27; −0.01]) when linear regression model was performed on *Pf* PD recorded during the last 3 months of follow-up (Table 6). The effect of *Sh* egg carriage was confirmed when the mean PD was dichotomized into classes using three different thresholds (Table 6).

4 | DISCUSSION

There is a lack of knowledge concerning the modulation of specific immune responses to *Pf* that occur in individuals with schistosomiasis. In our study, a modulation of antimalarial immunity and *Pf* PD

was observed in school-age Beninese children infected by *Sh*. A systematic review and meta-analysis performed by Degarege and coll. showed that the density of *Pf* infection was lower in children coinfected with *Sh* compared to those uninfected with *Sh*.[38]

We found that children infected by *Sh* had higher concentrations of various IgG isotypes directed to MSP3 and GLURP antigens compared with uninfected. Higher levels of IgG directed to $MSP1_{-19}$ and to crude *Pf* schizont extracts have previously been observed in *Sh-Pf* coinfected Senegalese children compared with those only infected by *Pf*.[20] However, two other studies found discordant results.[18,19] In a Senegalese cohort, Courtin and coll. found that levels of IgG directed to GLURP and MSP-1 were lower in *Sh*-infected children than in those uninfected.[19] More recently, Ateba-Ngoa and coll. showed that the level of IgG to the *Pf* sexual stage antigen Pfs48/45 was lower in Gabonese children infected by *Sh* compared with those uninfected. Of note, no association between *Sh* infection and Ab responses to asexual antigens such as GLURP were reported in that study.[18] Differences in methodologies used in these studies to quantify Ab responses, to detect infections or to determine the clinical and parasitological phenotypes could partly explain the observed variation in the modulation of *Pf*-specific Ab responses associated with *Sh* infection. We have ourselves nevertheless reported lower *Pf*-specific Ab responses in the presence of *Sh* in Senegalese children but higher levels of the same Ab responses in the presence of *Sh* in Beninese children using the same methodologies. Other factors such as age of study population or the duration and/or intensity of *Sh* infection could possibly explain the differing results observed in these two studies. Notably, the children included in the Senegalese study were older (6-19 years old) than those included in the present study (4-8 years old). We hypothesize that younger individuals have a more Th2-skewed immune responses, whilst older children have a stronger regulatory immune response that is characterized by cytokine production such as interleukin (IL)-10. Higher levels of IL-10 were observed in the *Sh*-infected versus uninfected Senegalese children, whilst no such difference was observed in the Beninese cohort studied here. Moreover, no difference in the frequency of regulatory T cells in *Sh*-infected and uninfected children was observed in the present study. The high concentrations of antimalaria antibodies associated with the absence of an increase in T regulatory cells in *Sh*-infected children are more in line with a Th2-skewed immune response than a regulatory immune response; however, the absence of difference in IL-4 concentration between groups of *Sh*-infected and uninfected children does not fully support our hypothesis.

The existence of Ab cross-reactivity between *Sh* egg antigens and *Pf* antigens is suspected. Therefore, the higher level of Ab observed in *Sh-Pf* coinfected individuals could be explained by a phenomenon of cross-reactivity.[21,39,40] The cross-reactivity observed was largely limited to the IgG3 isotype response.[40] However, Diallo and colleagues evaluated the cross-reactivity of IgG1 and IgG3 against *Pf* antigens using a procedure of adsorption of sera with total schistosome antigens and rejected the hypothesis of cross-reactivity. Moreover, the GLURP recombinant protein used in the present study was similar to that used in the Senegalese study in which lower Ab responses to GLURP were reported in the *Sh*-infected group.[19] This result argues against the involvement of cross-reactivity, at least for the GLURP recombinant protein.

TABLE 4 Effect of *Sh* infection on antibody responses (linear regression model)

	Multivariate analyses[a]		
Antibody concentration	**Lower/higher[b]**	**Coefficient [IC 95%]**	***P***
IgG1-MSP3	Higher	0.99 [0.35; 1.64]	.002
$IgG1\text{-}GLURP_{R0}$	Higher	0.76 [0.24; 1.29]	.005
$IgG2\text{-}GLURP_{R0}$	Higher	0.91 [0.35; 1.47]	.001
IgG3-MSP3	Higher	1.75 [1.03; 2.48]	<.001
$IgG3\text{-}GLURP_{R0}$	Higher	0.89 [0.26; 1.50]	.006
$IgG3\text{-}GLURP_{R2}$	Higher	1.54 [0.88; 2.20]	<.001

[a]The effect of *Sh* infection on anti-*Pf* Ab concentrations was adjusted on all co-variables associated with Ab modulation in univariate analyses (Table S2).

[b]The Ab concentrations were compared between children without *Sh* eggs and those with *Sh* eggs. The values of regression coefficient are positive that show higher anti-*Pf* Ab concentrations in children with *Sh* eggs.

TABLE 5 Characteristics of the children with samples included in flow cytometric analyses

Infection status	**Absence of *Sh* eggs (n = 25)**		**Presence of *Sh* eggs (n=25)**	
Groups	*Sh-/Pf-*	*Sh-/Pf+*	*Sh+/Pf-*	*Sh+/Pf+*
n	19	6	15	10
Male/female	12/7	4/2	9/6	7/3
Age mean (in years)	7	7	7	7
Ethnicity	Toffin	Toffin	Toffin	Toffin
Frequency of *Pf* infection in %	24% (6/25)		40% (10/25)	
Frequency of *Pf* febrile infection in %	100% (6/6)		88% (8/10)	
Parasitemia (iRBC/µL) [range]	0	31 238 [929-169990]	0	6409 [356-49315]

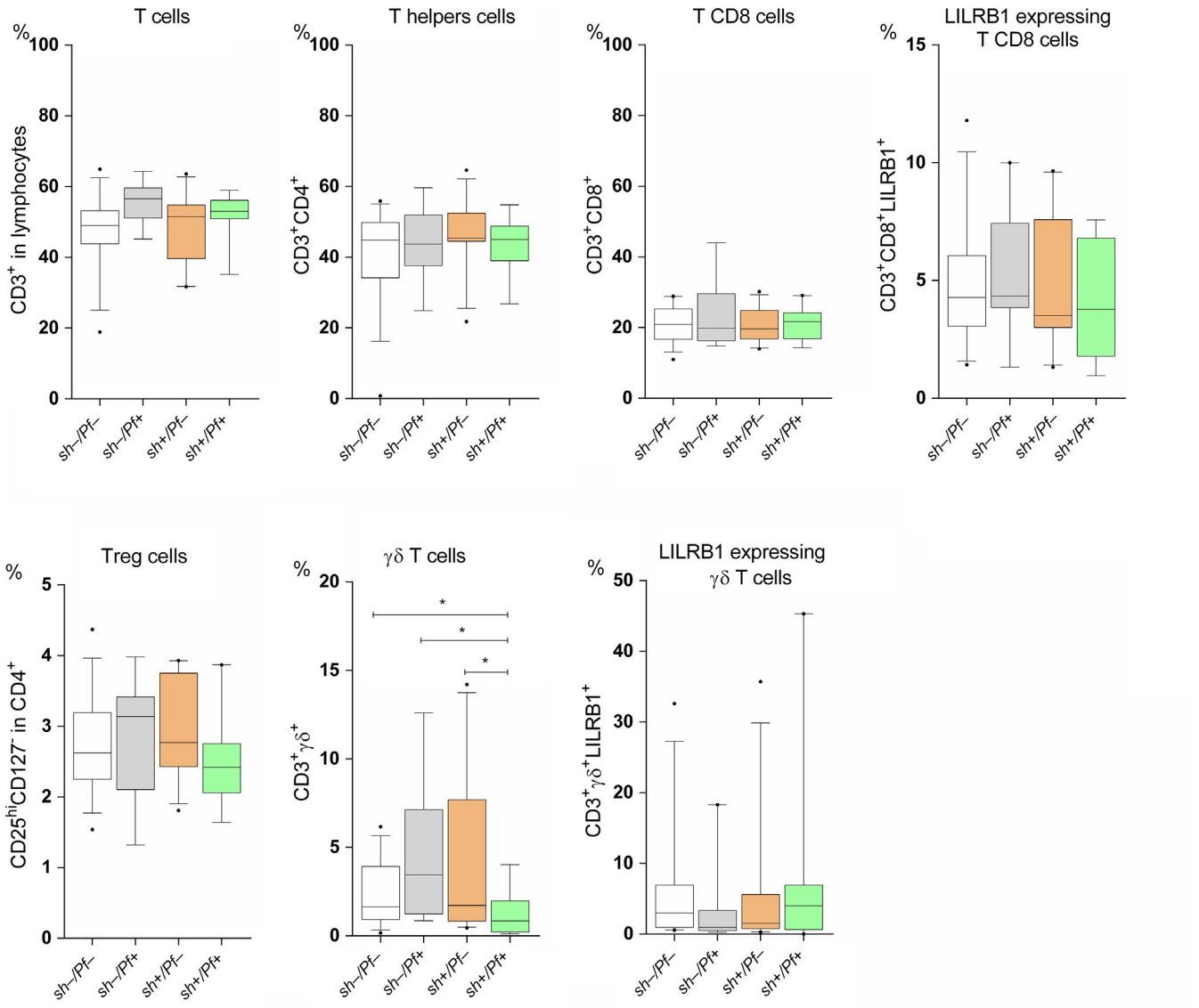

FIGURE 3 T-cell frequency according to the *Sh* and *Pf* infection status. Box-whisker plots represent medians with 25th and 75th percentiles (boxes) and with 10th and 90th percentiles (whiskers). The frequencies of T cells, T helper cells, CD8 T cells, Treg cells, γδT cells and the LILRB1 expression at the surface of CD8 T cells and γδT cells were compared between (a) uninfected children (*Sh*-/*Pf*-; n = 19), (b) children only infected by *Pf* (*Sh*-/*Pf*+; n = 6), (c) children only infected by *Sh* (*Sh*+/*Pf*-; n = 15) and (d) children coinfected by *Sh* and *Pf* (*Sh*+/*Pf*+, n = 10) using non-parametric Mann-Whitney test. * $P < .05$

No difference was observed in B-cell frequencies in the groups of interest. We were unable to include markers of B-cell activation or B-cell subpopulations such as regulatory B cells in our analyses due to technical constraints related to the blood volume collected and the limited capacity of the 4-colour flow cytometer used. The higher concentration of anti-*Pf* antibodies in children infected by *Sh* could result from differences in B-cell subpopulation proportions including higher amount of activated B cells and/or plasmocytes and lower amount of regulatory B cells. Our results did show that children coinfected by *Pf* and *Sh* had lower frequencies of γδ T cells than those only infected by *Pf*. The higher levels of IFN-γ observed in *Pf*-infected individuals would be consistent with a higher frequency of γδ T cells since such cells are well-recognized IFN-γ producers.[41] Expansion of γδ T cells during *Pf* infection has been observed in humans and in mouse models, but the role of γδ T cells in protection against blood stage *Pf* remains an open question.[42]

HLA-G can modulate the activity of the major cell components of the innate and adaptive immune responses.[23] Therefore, we have investigated the role played by HLA-G, and its receptors, in the regulatory mechanisms expected during *Sh* infection. No difference in soluble HLA-G concentration was observed according to the *Sh* infectious status in our study. However, the LILRB2 expression was lower at the surface of neutrophils from children coinfected than those uninfected. Neutrophils provide defence against *Pf* through different mechanisms such phagocytosis, production of reactive oxygen species or Ab-dependant respiratory burst.[43] Functional assays indicate that after engagement with LILRB2 HLA-G inhibits the phagocytic function of neutrophils.[44] In addition, LILRB2 ligation

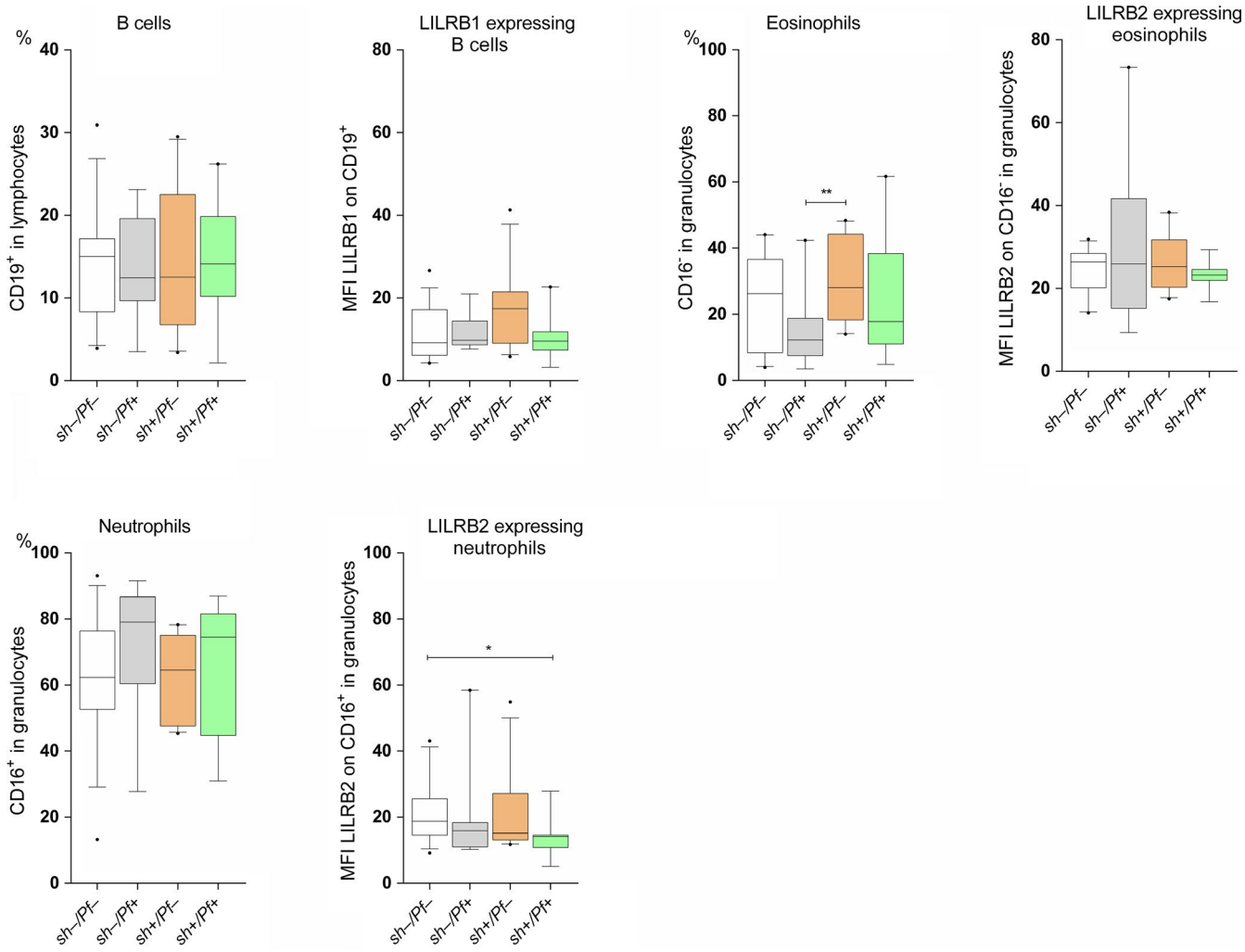

FIGURE 4 Frequencies of B cells, eosinophils and neutrophils according to the *Sh* and *Pf* infection status. Box-whisker plots represent medians with 25th and 75th percentiles (boxes) and with 10th and 90th percentiles (whiskers). B cell, eosinophil and neutrophil frequencies and the LILRB1 and LILRB2 expression at their surface were compared between (a) uninfected children (*Sh*-/*Pf*-; n = 19), (b) children only infected by *Pf* (*Sh*-/*Pf*+; n = 6), (c) children only infected by *Sh* (*Sh*+/*Pf*-; n = 15) and (d) children coinfected by *Sh* and *Pf* (*Sh*+/*Pf*+, n = 10) using non-parametric Mann-Whitney test. * $P < .05$

impairs production of reactive oxygen species induced by FcγRIIa.[45] The lower expression of LILRB2 observed at the surface of neutrophils in *Sh* eggs carriers could correspond to a higher capacity of neutrophils to neutralize *Pf* directly via phagocytosis or indirectly via the fixation of Ab on their Fcγ receptors.

No association was found between *Sh* egg carriers at the end of the follow-up and the prevalence of *Pf* infection in our study; however, lower mean *Pf* PD in *Sh*-infected children was observed during the follow-up and at the end of the study. Unfortunately, helminth diagnosis was not systematically performed every month like malaria diagnosis; therefore, it was not possible to know precisely when the children were infected during the follow-up. Therefore, some *Pf* parasitemias included in the calculation of mean parasite density during the follow-up in the *Sh*-positive group were likely detected when the children were not yet infected by *Sh*. Such misclassification may increase the mean of *Pf* parasitemia in the *Sh*-positive group making the identification of associations between *Sh* status and *Pf* mean parasite density more difficult. We have performed a complementary analysis on the last 3 months of follow-up to cover the prepatent period of *Sh* (10-12 weeks), and the association was confirmed ($P = .016$; lower mean parasite density in the *Sh*-infected group).

Sh has been reported to modulate malaria PD in Senegalese children, in which those with light intensity *Sh* infections had lower *Pf* PD than *Sh* uninfected children.[3,5,38] In our study, the effect of *Sh* infection on *Pf* PD seemed to be independent of the intensity of *Sh* infection because no difference in *Pf* PD was observed when *Sh* infection was dichotomized into low, moderate or heavy *Sh* intensity. Another study conducted in Mali indicated that *Sh* infection was associated with lower *Pf* PD and a reduced risk of progression to symptomatic disease in long-term *Pf* asymptomatic carriers.[7] However, others found that *Sh* infection increases the risk of *Pf* infection and the prevalence of *Pf* parasites in *Sh*-infected children.[8] The risk of *Pf* infection was clearly increased when *Sh* infection was combined

TABLE 6 Effect of *Sh* infection on *Pf* parasite densities

Multivariate analyses[a]				
	***Pf* parasite density (PD)[a]**	**Effect of *Sh* infection on *Pf* PD**	**Coefficient [IC 95%]**	***P***
Linear regression (Model 1)	Quantitative	Lower *Pf* PD	−0.87[−1.41; −0.33]	.002
Linear regression (Model 2)	Quantitative	Lower Pf PD	−0.64 [−1.27; −0.01]	.045
Logistic regression[b]	3.980 (median)	Lower *Pf* PD	−0.99 [−1.64; −0.34]	.003
	2.500	Lower *Pf* PD	−1.01 [−1.66; −0.37]	.002
	5.000	Lower *Pf* PD	−1.04 [−1.72; −0.36]	.003
	10.000	Lower *Pf* PD	−0.96 [−1.73; −0.18]	.015

Note: The effect of *Sh* infection on the mean number of iRBC/8000 leucocytes was investigated using linear regression models after adjustment on factors influencing *Pf* PD such as sickle cell trait, age and Ab concentrations. Linear regression model was performed on *Pf* PD recorded during the whole follow-up (model 1) or on *Pf* PD recorded during the last 3 month of follow-up (model 2).

[a]Pf parasite density expressed in infected Red Blood Cells/8000 leucocytes.

[b]The logistic regression models were used to assess the role of *Sh* infection on parasite density according to different thresholds. The *Pf* parasite density was dichotomized in qualitative variables as follows: (a) < and ≥ to the median value (3980 iRBC/8000 leucocytes); (b) < and ≥ to 2500 iRBC/8000 leucocytes; < and ≥ to 5000 iRBC/8000 leucocytes and finally < and ≥ to 10 000 iRBC/8000 leucocytes.

T trichiura or hookworm infection.[8] In our study, no *T trichiura* infection was detected in the *Sh*-infected children whilst only two *Sh*-negative children were infected by *T trichiura*. No other STH species were detected in either groups; therefore, infection by STH was not a confounder in our study. The low prevalence of STH infection was probably related to unfavourable environmental factors. The transmission cycle of STH seems to be impaired when children live in stilt houses built on lake Nokoué.

In conclusion, our study demonstrates that the presence of *Sh* modulates host immune responses directed to *Pf*. The higher concentration of antimalarial Ab responses observed in *Sh* egg carriers may result from a complex phenomenon of activation, suppression and regulation of immune pathways. The pattern of immune down-regulation usually reported during helminth infection was not observed in our study. We hypothesize that, in young children such as those studied here, the relatively recent acquisition of *Sh* infections compared with the older children in our Senegalese study may partly explain the absence of a strong regulatory component of the immune response that we observed in the latter study. Modulation of immune responses to *Pf* during helminth infection is complex but is real and does influence the development of *Pf* during co-infections. Programmes of mass drug administration for control of helminths will have the direct advantage of killing worms but will certainly have consequences in the context of immune responses that may be advantageous depending on the particular pathogen and/or pathological status of those treated. We suggest that it will be important to follow school-aged children in areas covered by repeated anti-helminthic mass drug administration in order to assess the consequences in terms of malaria epidemiology and immune response.

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CONFLICT OF INTEREST

All the authors declared no conflict of interest.

AUTHOR CONTRIBUTIONS

AM, AG, MI and DC conceived, designed and coordinated the study. LT, ON, PS, GM, SG, PA and DG participated to the sample collection and processing. BF, AL, ED and DC designed and supervised the immunoassays. TT and AA supervised the medical follow-up. LT, JM, AG and DC performed statistical analysis. LT, ON, PS, GM, SG and PA carried out the immunoassays. LT, ON, MI and DC drafted the first version of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Data available on request due to ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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