HELMINTH INFECTIONS ARE ASSOCIATED WITH PROTECTION FROM CEREBRAL MALARIA AND INCREASED NITROGEN DERIVATIVES CONCENTRATIONS IN THAILAND

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Abstract. Following a study showing an association between *Ascaris* and protection from cerebral malaria, we hypothesized helminths may have induced protection through immunoglobulin E (IgE) and the CD23/NO pathway. We compared the prevalence of helminth infections in 67 cerebral malaria patients and 217 hyperparasitemic controls with no complications. For 24 cerebral malaria cases and 56 controls, we compared reactive nitrogen intermediates (RNI) concentrations and their correlations to total IgE and sCD23 concentrations in helminth-infected and noninfected patients. We observed a dose-dependent association between helminth infections and protection from cerebral malaria (adjusted odds ratio [OR] = 0.36, 95% CI = 0.19–0.7, *P* = 0.002, linear trend *P* = 0.0007). Helminth-infected controls had higher RNI concentrations than those without helminths: 72 OD ± 19 SD and 57 OD ± 20 SD, respectively (*P* = 0.006). Logistic regression, including interaction terms between RNI and sCD23, showed that an increase of RNI could be both protective and pathogenic depending on the concentration of sCD23. Helminths increasing both the CD23 receptor and its ligand may have a role in the establishment of malaria tolerance through the CD23/NO pathway.

INTRODUCTION

*Plasmodium falciparum* still kills > 1 million people every year. Although in many endemic areas nearly everyone contracts *falciparum* malaria, only a minority of patients (~1%) will develop cerebral malaria. The virulence of the infecting strain appears to be an important factor, but it has also been shown that immunogenetic or acquired host factors are determinants. In a 2000 study, we observed a dose-dependent association between *Ascaris lumbricoides* and protection from cerebral malaria in humans. Other helminths such as hookworms or *Trichuris trichiura* increased the protection related to *Ascaris*. In the tropics, a majority of young children are infected by intestinal helminths. Therefore, at a population level, the significance of such protection could be considerable. The role of nitric oxide (NO), a major immune effector in malaria, described both as pathogenic and protective, is unclear. Technical difficulties linked to the short half-life of the radical and the impossibility of correlating peripheral measurements to *in situ* production are possible explanations. These discrepancies could also reflect differences in background immunity or in NO induction processes. Both helper T1—through interferon-gamma (IFN-γ)—and helper T2 lymphocytes-through induction of the FcγRII/CD23 receptor and its ligand immunoglobulin E (IgE)—can induce type 2 NO synthase (iNOS), which generates large quantities of NO. Helminths usually shift the immune profile toward a Th-2 response, increasing CD23 expression and IgE concentrations. Therefore, during malaria, we hypothesized that preexisting helminth infections may induce the CD23/NO pathway. Our objectives here were to (1) determine whether there were any significant differences in NO derivatives between helminth-infected and helminth-non-infected patients and correlate them with the total IgE concentrations and the soluble fraction of the CD23 receptor; and (2) determine whether differences in the equilibrium between sCD23 and NO derivatives could predict the risk of cerebral malaria.

PATIENTS AND METHODS

**Study site.** The study was performed at the Hospital for Tropical Diseases, Bangkok, Thailand. It is the national referral hospital for malaria, notably for severe malaria and cerebral malaria with high parasite biomass. The study was approved by the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University, and all patients gave informed consent.

**Inclusion and exclusion criteria.** Between January 1998 and December 1999, we included 67 consecutive patients with cerebral malaria as defined by a neurological dysfunction (Glasgow Coma Score < 10) and 217 controls, defined by the absence of any of the 10 World Health Organization defining criteria despite a high *P. falciparum* parasite biomass (parasitemia > 5% and/or 200,000/µl and/or presence of schizonts on the peripheral blood smear).

In a subgroup of 80 consecutive patients observed between January and October 1998, we collected a blood specimen to measure reactive nitrogen intermediates (RNI). Patients with acute renal failure were excluded because it would have interfered with RNI measurements.

Patients with glucose-6-phosphate dehydrogenase deficiency, hemoglobinopathies, and mixed *P. falciparum–P. vivax* infections were excluded.

**Choice of controls.** It has been suggested that, at a population level, the relative rarity of cerebral malaria reflects that of the most virulent strains. In this perspective, the best control group to study host factors related to cerebral malaria would have been patients infected by the same strain who did not develop cerebral malaria. In the absence of known stable virulence markers, and because there is a positive correlation between parasite biomass and complications, we chose controls with a high parasite biomass and adjusted for symptoms duration. Patients hence had parasites of comparable growth kinetics and only differed from the clinical status reflecting presence or absence of cerebral dysfunction. Thus, after tak-
ing parasite biomass in account and adjusting for time, we assume this reduced the specific effect of the strain, thereby facilitating the detection of host-related protective/risk factors. A structured questionnaire on background data such as malaria history, residence duration in the endemic area, and socioeconomic showed that, in addition, in our setting, choosing controls with no complications despite a high parasite biomass allows reduction of the referral hospital bias.15

Exposure. Different helminths have in common the capacity to increase IgE levels. Therefore, we grouped them in one single exposure variable: presence of helminths.

Variables. On admission patients were examined, and for 24 cases and 56 controls a 5-ml blood sample was drawn and plasma was then frozen at −40°C. We measured reactive nitrogen intermediates (optical density)‡ between helminth-infected and non-helminth-infected controls.19

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cerebral malaria</th>
<th>Controls</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median parasitemia (/µl)</td>
<td>271,680</td>
<td>251,840</td>
<td>P = 0.9*</td>
</tr>
<tr>
<td>Helminth infections (n (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>46 (69)</td>
<td>96 (44)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21 (31)</td>
<td>121 (56)</td>
<td></td>
</tr>
<tr>
<td>Heavy worm burden</td>
<td>0 (0)</td>
<td>5 (2)</td>
<td></td>
</tr>
<tr>
<td>Medium worm burden</td>
<td>6 (9)</td>
<td>38 (17)</td>
<td></td>
</tr>
<tr>
<td>Low worm burden</td>
<td>15 (22)</td>
<td>78 (37)</td>
<td></td>
</tr>
<tr>
<td>Reactive nitrogen intermediates (optical density)§§</td>
<td>71 ± 22 (6)</td>
<td>72 ± 198§ (23)</td>
<td>P = 0.16#</td>
</tr>
<tr>
<td>Helminths</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No helminths</td>
<td>70 ± 21 (17)</td>
<td>57 ± 20 (33)</td>
<td></td>
</tr>
<tr>
<td>Grouped</td>
<td>71 ± 21</td>
<td>64 ± 21</td>
<td></td>
</tr>
<tr>
<td>Total IgE, geometric mean**</td>
<td>3,071 (6)‡‡</td>
<td>2,930 (19)‡‡</td>
<td>P = 0.9#</td>
</tr>
<tr>
<td>Helminths</td>
<td>1,118 (12)</td>
<td>963 (24)</td>
<td>P = 0.7†</td>
</tr>
<tr>
<td>No helminths</td>
<td>1,578</td>
<td>1,630</td>
<td>P = 0.9#</td>
</tr>
<tr>
<td>Grouped</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCD23, geometric mean§§</td>
<td>2,121 (6)††</td>
<td>1,754 (21)##</td>
<td>P = 0.7#</td>
</tr>
<tr>
<td>Helminths</td>
<td>2,591 (16)</td>
<td>1,939 (26)</td>
<td>P = 0.3#</td>
</tr>
<tr>
<td>No helminths</td>
<td>2,465</td>
<td>1,958</td>
<td>P = 0.3#</td>
</tr>
</tbody>
</table>

* Two-sample Wilcoxon’s rank sum test.
† Adjusted for age, body mass index, mean corpuscular volume, and duration of symptoms using unconditional logistic regression.
‡ Values represent means ± standard deviations. Available observations = 79.
†† The difference between helminth-infected and non-helminth-infected controls was significant (Student’s unpaired t-test P = 0.006).
§ In the control group, total IgE concentrations were adjusted using multiple linear regression with RNI as a dependent variable and helminths and IgE as independent variables. The association between helminths and RNI disappears (P = 0.6) after adding IgE in the model (P = 0.02).
# Student’s unpaired t-test.
** Values represent international units. Available observations = 61.
‡‡ Comparison of logIgE: between helminth-infected and non-helminth-infected cerebral malaria cases using Student’s unpaired t-test (P = 0.05).
## Comparison of logIgE: between helminth-infected and non-helminth-infected controls using Student’s unpaired t-test (P = 0.001).
††† Comparison of logIgE: between helminth-infected and non-helminth-infected cerebral malaria cases using Student’s unpaired t-test (P = 0.7).
variables in four different groups: cases with helminths, cases without helminths, controls with helminths, controls without helminths.

To explain the variance of RNI in these groups, we used multiple linear regression models. The best model was chosen according to its adjusted $R^2$ value. The frequency of helminth infections among cases and controls allowed us to obtain crude odds ratios. Using an unconditional logistic regression model, we adjusted for the following potential confounders: duration of symptoms, age, body mass index (weight/height$^2$) (to adjust for the nutritional status), and the mean corpuscular volume (to adjust for iron deficiency, which leads to microcytosis). Statistical significance was reached for $P < 0.05$.

Hypothesizing that the CD23–NO relationship was a key factor for the interpretation of RNI concentrations and for the outcome of malaria, we used the following logit function:

$$\log\left(\frac{\text{probability of disease}}{1 - \text{probability of disease}}\right) = \beta_0 + \beta_1 \cdot (\text{RNI concentration} \cdot X_1) + \beta_2 \cdot (\text{scCD23 concentration} \cdot X_2) + \beta_3 \cdot (\text{RNI concentration} \cdot \text{scCD23 concentration} \cdot X_2).$$

To evaluate the interaction between RNI concentrations and scCD23, we calculated the logit function for a 10-unit increase in RNI concentrations.

$$\text{OR}_{\text{stratum}} = \exp\{\beta_1 + 10 \cdot \beta_2 + \beta_3 \cdot \text{scCD23 concentration} \cdot X_2\}.$$  

The stratum-specific odds ratio for a 10-unit increase in RNI concentrations was calculated for a 10-unit increase in RNI concentrations, for a fixed scCD23 concentration $X_2$. The logit function became:

$$\log\left(\frac{\text{probability of disease}}{1 - \text{probability of disease}}\right) = \beta_0 + \beta_1 \cdot (\text{RNI concentration} + 10 \text{ units}) + \beta_2 \cdot (\text{scCD23 concentration} \cdot X_2) + \beta_3 \cdot (\text{RNI concentration} + 10 \text{ units}) \cdot \text{scCD23 concentration} \cdot X_2.$$  

The stratum-specific odds ratio for a 10-unit increase in RNI concentrations (ARNI) was obtained with the ratio between the two formulas just given, which finally led to:

$$\text{OR}_{\text{ARNI}} = \exp\{10 \cdot \beta_2 + \beta_3 \cdot \text{scCD23 concentration} \cdot X_2\}.$$  

Using this formula for different concentrations of scCD23 allowed us to illustrate the evolution of the odds ratio associated with a 10-unit increase in RNI concentrations.

**RESULTS**

**General data.** Patients were a median age of 27 years (interquartile range [IQR] = 22–34) and controls 25 years ($P = 0.3$). The sex ratio was equivalent in cases and controls (85% males). The median symptoms duration was longer among cases (5 days [IQR = 4–9]) than among controls (4 days [IQR = 3–6]), but this was not significant ($P = 0.12$). Fifty-seven per cent of cases and 46% of controls were of Thai ethnicity ($P = 0.26$).

**Odds ratio.** Comparing the prevalence of helminth infections between cases and controls and adjusting for symptoms duration, age, body mass index, and mean corpuscular volume showed a 64% protection (1 – odds ratio) from cerebral malaria (see Table 1). The trend chi-square showed a dose-response relationship between helminth quantity and protection ($P = 0.0007$) (see Table 1). Similarly, there was a linear trend between the number of different parasites and protection ($\chi^2 = 10.7, 1 \text{ df, } P = 0.001$).

**Arguments for reduced sequestration of parasitized red blood cells in helminth-infected patients.** For controls, the median ratio between circulating schizonts and parasitemia—a reflection of the maturity of parasites and thus of the proportion of sequestered parasites—was significantly lower in helminth-infected controls: 1 schizont per 10,000 trophozoites (IQR = 0–5) relative to 3 schizonts per 10,000 trophozoites (IQR = 0.3–13) ($P = 0.04$). Among controls RNI concentrations were negatively correlated with the schizont/trophozoite ratio ($n = 56$, Spearman's rho = −0.28, $P = 0.038$).

On the contrary, among cases, helminth-infected cerebral malaria cases with circulating schizonts had a higher median proportion of mature forms—38 schizonts per 10,000 trophozoites (IQR = 14–52)—than cerebral malaria cases without helminths: 6 schizonts per 10,000 trophozoites (IQR = 1–26) ($P = 0.02$). The geometric mean duration of symptoms was longer in helminth-infected patients (6.9 days) than in patients without helminths (4.9 days) ($P = 0.045$).

**Helminths, IgE, and RNI.** There was no significant difference for the mean RNI and IgE concentrations between cerebral malaria patients and controls (see Table 1). Among controls the mean RNI concentrations were significantly higher in helminth-infected relative to patients without helminths (see Table 1). Significance remained after adjustments for age, creatininemia, and evolution duration ($P = 0.001$), but after adjustment for IgE concentration the association disappeared (see Table 1). There were no massive worm infections among the patients from whom we obtained a blood sample, but mean RNI concentrations seemed to increase with the worm burden: mean RNI concentration for medium worm burden 80.6 ± 17 OD ($n = 5$) versus 70 ± 19 OD ($n = 24$) for patients with a low worm burden and 61 ± 21 OD ($n = 50$) for patients with no helminths ($P = 0.06$).

**RNI, IgE, and scCD23 correlations.** Table 2 shows the analysis of the relation between RNI, total IgE, and scCD23 stratified by the presence or absence of neurological signs and the presence or absence of helminths. The results show a positive correlation between RNI and IgE in helminth-infected patients. This correlation was absent in patients without helminths. In addition, Table 2 shows that all controls had a negative correlation between RNI and scCD23 and that, on the contrary, helminth-infected cerebral malaria cases had a significant positive correlation between RNI and scCD23; however, the number of patients was small ($n = 6$).

**Multiple linear regression models predicting RNI concentrations.** Table 3 shows that for controls with helminth infec-

<table>
<thead>
<tr>
<th>Variable</th>
<th>RNI/logIgE</th>
<th>RNI/logCD23</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>r</td>
</tr>
<tr>
<td>Cerebral malaria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helminths</td>
<td>6</td>
<td>0.92</td>
</tr>
<tr>
<td>No helminths</td>
<td>12</td>
<td>0.06</td>
</tr>
<tr>
<td>Nonsevere malaria with high parasite biomass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helminths</td>
<td>19</td>
<td>0.68</td>
</tr>
<tr>
<td>No helminths</td>
<td>24</td>
<td>−0.09</td>
</tr>
</tbody>
</table>
HELMINTHS AND FALCIPARUM MALARIA

DISCUSSION

The dose-dependent association between helminths and protection from cerebral malaria, the preexistence of helminth infections to malaria, and the existence of a similar animal model\textsuperscript{17} led us to favor a causal relationship between helminths and protection from cerebral malaria. Helminth-infected controls seemed to have a lower proportion of peripheral mature schizonts than controls without helminths, suggesting the first group had fewer sequestered parasites.\textsuperscript{13} On the contrary, cerebral malaria with helminths had a higher proportion of mature schizonts than cerebral malaria without helminths, suggesting that helminth-infected patients required a higher sequestered parasite biomass to develop cerebral malaria. Two possible confounders could have been the poor nutritional and socioeconomic status\textsuperscript{5,7} associated with helminths. Here, adjustments for the body mass index...
A possible explanation for the lower peripheral RNI concentrations in controls without helminths would be that the baseline expression of membrane CD23 was lower than in helminth-infected patients, who have high concentrations of interleukin-4, a potent inducer of CD23 and one of its ligands: IgE.

On the contrary, the findings in cerebral malaria without helminths suggested that NO did not result from CD23 ligation alone. A possible alternative could have been a post-hoc induction of INOS in response to Th-1 cytokines. However, given the small number of helminth-infected cerebral malaria cases, correlations in that group should be reconfirmed despite their significance.

In studies using different comparison groups, total IgE elevation has been described as a pathogenic factor during malaria. We did not find any significant difference between total IgE concentration in cerebral malaria and our controls. This suggests that the ability of IgE immune complexes to induce tumor necrosis factor-ga may not be equivalent to cerebral pathology. The comparison of severe malaria cases with mild malaria may have contributed to the observation of significant differences in IgE concentrations because of major differences in total parasite biomass between these groups.

Altogether, these results are compatible with the hypothesis of the IgE/CD23/NO-mediated reduction of cytoadherence protecting helminth-infected patients. This was further supported by an in vitro study on human lung endothelial cells showing that CD23 ligation induced NO release and reduced ICAM-1 expression and cytoadherence of parasitized red blood cells (Traore and others, unpublished data).

In populations widely infected by helminths, the selective force of malaria may have shaped the most successful immune responses against malaria in hosts presenting a shift toward Th-2 cytokines. In this perspective, helminth infections, because of their high prevalence in young children, may be an important element in the acquisition of malaria tolerance by increasing both the CD23 receptor and one of its ligands, IgE complexes. Assuming there are > 1 million deaths per year and that between 30% and 80% of the population at risk of cerebral malaria also carries helminths and considering the observed 64% protection, this implies that each year, between at least 192,000 and 512,000 deaths from cerebral malaria are avoided by helminths. Helminths are indeed associated with morbidity and sometimes mortality but not in proportions comparable to malaria. They also may be detrimental in diseases such as tuberculosis and HIV. Nevertheless, the prior calculation inspires caution in systematic antihelminthic treatment, at least in groups in which cerebral malaria is still a major killer. This risk may not only be theoretical, as suggested by the increase of malaria attacks after treatment of Ascariasis.

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