HIGH TITERS OF IgG ANTIBODIES AGAINST PLASMODIUM FALCIPARUM SERINE REPEAT ANTIGEN 5 (SERA5) ARE ASSOCIATED WITH PROTECTION AGAINST SEVERE MALARIA IN UGANDAN CHILDREN

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Abstract. Plasmodium falciparum serine repeat antigen (SERA5) is a promising asexual blood stage malaria candidate vaccine. However, there is a paucity of information about natural immune responses to SERA5 in children from malaria-endemic regions. We undertook a hospital-based case-control study of severe malaria in Apac District, Northern Uganda, in children 6–59 months of age. The commonest symptoms observed in children with severe malaria (SM) were respiratory distress (53.4%) and prostration (40.4%) followed by circulatory collapse (7.4%), severe anemia (Hb < 5 g/dL, 7.0%), and seizures (2.6%). None of the SM children had impaired consciousness, coma, or cerebral malaria. We measured serum IgG antibodies using a recombinant construct of SERA5 (SE36) in enzyme-linked immunosorbent assays. High titers of IgG anti-SE36 were associated with protection against severe malaria in children under 5 years old.

INTRODUCTION

In Uganda, malaria poses a formidable burden to an over-stretched and underfunded public health system trying to cope with other pressing health problems like HIV/AIDS and tuberculosis. Twenty-five to thirty percent of all outpatient visits and 300 infant deaths daily are due to malaria alone, not to mention the significant proportion of low birth weight deliveries due to malaria during pregnancy. The problem is exacerbated by the emergence of drug-resistant Plasmodium falciparum strains and insecticide-resistant Anopheles gambiae mosquito vectors. The development and clinical application of efficacious malaria vaccines is therefore an urgent imperative. One promising vaccine candidate is Plasmodium falciparum serine repeat antigen (SERA5). A recombinant amino terminal fragment of SERA5 induces protective immune responses against P. falciparum infection in Aotus monkeys. After immunization of rats and Saimiri monkeys, SE47' induced antibodies that inhibited parasite growth in vitro and protected monkeys against challenge infection, respectively. Ugandan adult sera with high titers of IgG3 anti-SE47' inhibited parasite growth. Recombinant constructs of SERA5, SE47', and SE50A, representing the amino terminus and central domains, respectively, have been evaluated for antigenicity in residents of Atopi Parish in Apac District in Northern Uganda. Recombinant SE47' is an expression product of a synthetic gene with Escherichia coli codons that encodes the amino terminal domain of SERA5 (amino acids 17 to 382). IgG3 and IgG1 antibodies were the predominant IgG subclass responses to SE47' and SE50A, respectively, in serum samples collected from adults and children during a cross-sectional study. The ability of individual Apac adult sera to inhibit P. falciparum growth in vitro was a function of the titer of IgG3 anti-SE47' antibodies. IgG3 anti-SE47' high responder parasitemic children aged about 6 years old were more likely to be afebrile on the day of sampling than low responders, suggesting that IgG3 anti-SE47' antibodies might be associated with clinical immunity in Apac children. This possibility has been investigated in a hospital-based case-control study of severe malaria in Apac children 6–59 months of age using SE36, another recombinant construct of SERA5 that is almost identical to SE47'.

We tested the hypothesis that high titers of natural IgG antibodies against a recombinant construct representing the amino terminus of SERA5 protects Ugandan children under 5 years old against severe malaria. The recombinant protein SE36, which is identical to SE47' but lacks the run of serine repeats, was used as antigen in enzyme-linked immunosorbent assays (ELISA). SE36 was recognized by sera from a considerable proportion of children, and antibody titers were associated with protection from some of the manifestations of severe malaria.

MATERIALS AND METHODS

Study area and subjects. This study took place in the Pediatric Ward and the Outpatient Department of Apac Hospital, Apac Town, Apac District, Northern Uganda. Apac Town is located about 300 km from Kampala, the capital city of Uganda. Apac District covers an area of 6,684 sq km and ranges in altitude between 1,350 and 1,500 m above sea level. The average annual rainfall is 1,350 mm and the average temperature is 27°C. The vegetation consists mainly of savannah woodland interspersed with a wide network of swamps. The major mosquito vectors are Anopheles gambiae and Anopheles funestus. About 98% of the population belongs to the Nilotic Langi tribe who practice subsistence agriculture and fishing to a large and small extent, respectively. There are two major rainy seasons from March to May and from July to August, which coincide with the peaks of malaria transmission. Our previous study showed that malaria transmission in Atopi Parish is hyperendemic and that acquired antiparasite immunity appears by 7 years of age. Children with severe and complicated malaria were recruited from the Pediatric Ward, whereas children with uncomplicated malaria were recruited from the Outpatient Department. Most of the children came from villages that are within a 10-km radius of the hospital.

Case-control study. The study design was a matched case-control study comparing antibody titers in serum samples of
children admitted to the Pediatric Ward with severe and complicated malaria (cases) on one hand, and children attending the Outpatient Department for unrelated illnesses but having parasitemia with no signs of severe disease requiring hospitalization (controls) on the other hand. The study population was children 6–59 months old who were attending or admitted to Apac Hospital with a primary diagnosis of malaria. Ethical approval was obtained from the Uganda National Council for Science and Technology. Informed consent was obtained from the parents/guardians, a standard questionnaire was used to collect information about each child’s clinical history and the use of antimalarial drugs and protective anti-mosquito measures, and a physical examination was carried out by a physician. Children with severe and complicated malaria (SM) were those hospitalized with fever and P. falciparum asexual parasitemia above > 10,000 parasites/μL, with one or more of the following conditions: severe malarial anemia (hematocrit < 20%, haemoglobin < 10 g/dL), respiratory distress (deep breathing), prostration, coma, multiple convulsions, renal failure, bacteraemia, hypoglycaemia, and others including jaundice, circulatory collapse, abnormal bleeding, and hemoglobinuria. The differential diagnoses for severe malaria that were considered and excluded were septicaemia, typhoid fever, pylonelphritis, lobar pneumonia, viral hepatitis, meningitis, encephalitis, Reyes syndrome, and drug effects/poisoning. Control subjects with uncomplicated malaria (UM) were children with either a positive parasitemia of ≤ 5,000 parasites/μL and temperatures above 37.5°C but who did not manifest any of the above signs of complicated malaria, or children with a positive parasitemia of ≤ 5,000 parasites/μL but who had neither fever nor any signs of complicated disease. The controls, matched for age, sex, and geographic location or parish residence, were recruited from the Outpatient Department when they presented with other illnesses and were well enough to go home. Inclusion criteria included willingness of the parent/guardian to provide informed consent and meeting the clinical criteria described above. Exclusion criteria included age under 6 months or above 60 months, life-threatening illnesses and clinical signs of pneumonia, bacterial or parasite-related gastroenteritis, HIV/AIDS-related opportunistic infections, and refusal by the parent/guardian to provide informed consent. Twomillilitre venous blood samples were collected from both cases and controls into clean tubes containing sodium citrate, and, after coagulation in a refrigerator for several hours, serum was separated from red cell pellets by centrifugation at 1,000 x g. All serum samples were stored at −20°C or −80°C until analyzed. Thick and thin blood smears on glass slides were made for each child. In all, 100 case/control-matched pairs were recruited with 5 unpaired subjects (3 with mild malaria and 2 with severe malaria) during a malaria transmission season between May and September 2002.

Microscopy. Thick and thin smears were stained with 3% Giemsa for 30 minutes. The thick films were quickly screened for the presence of parasites and an estimate of parasitemia so that children with confirmed malaria diagnosis were promptly treated with antimalariais. Thin films were examined later in the research laboratory for the speciation of malaria parasites using morphologic characteristics and quantification of parasitemia. The parasite density per microliter of blood was calculated from the number of parasites per 200 white blood cells (WBC), assuming an average normal WBC count of 8000/μL. A blood slide was declared negative after reading 100 high-power fields. Quantification of pigmented white blood cells was by microscopy. A total of 500 WBC were examined for the presence of pigment. The data were expressed as the number of pigmented WBC for every 500 WBC counted, as the proportion of pigmented WBC per slide, and as the proportion of children with pigmented WBC.

Enzyme-linked immunosorbent assay. Three antigens were used, namely, SE36, SE50A, and a total P. falciparum schizont lysate. Synthetic genes encoding SE47 and SE50A representing the amino terminus and central domains, respectively, of SERA5 were constructed using Escherichia coli codons and the recombinant proteins were expressed in E. coli. The synthetic gene construct encoding the recombinant antigen SE36 was identical to that encoding SE47 except that the 105-bp nucleotide sequence encoding a run of 35 serines were removed. The resulting SE36 is easier to purify and re-fold at a higher yield. The total P. falciparum schizont lysate was made by rupturing mature Percoll-separated schizonts by repeated freezing on dry ice and thawing on water at 37°C. The optimal antigen concentrations of 1.0 μg/mL for SE36 and SE50A, and a 1:1000 dilution for P. falciparum schizont lysate (protein content 2 μg/ml) were determined by checkerboard titration and used in direct ELISA. All secondary antibodies used were conjugated to horseradish peroxidase (Dako Ltd, High Wycombe, UK). Flat-bottomed 96-well Immunolon 4 plates (Dynatech, Billingshurst, UK) were coated overnight at 4°C with 100 μL of antigen at a concentration of 1 μg/mL in carbonate coating buffer, pH 9.6. The plates were washed 3 times in PBS/Tween 20 (PBS/T) and blocked with 1% bovine serum albumin (BSA) or 1% skimmed milk powder in PBS/T for 3 hours at room temperature. The plates were again washed 3 times with PBS/T. The test sera were added (100 μL per well) at dilutions of 1:1000 in 1% BSA in PBS/T and the plates incubated overnight at 4°C. After the plates were washed 4 times in PBS/T, horseradish peroxidase conjugated anti-human IgG diluted 1:1000 in PBS/T was added and incubated at 37°C for 1 hour. The plates were washed 4 times, o-phenylenediamine (OPD) substrate (Sigma Chemical Industries, Inc, St. Louis, MO) was added, and the plates developed for 10 minutes. The reaction was stopped with 25 μL of 2 M sulfuric acid and the plates read at 492 nm. Titer of IgG anti-SE36 were estimated by titrating individual subject sera from 1:500 to 1:128,000 and selecting the serum dilution that gave an OD higher than that of the European negative controls plus 2 standard deviations on ELISA. The European controls were a set of sera from Europeans who had never been exposed to malaria. The prevalence of IgG anti-SE36 or total P. falciparum schizont lysate was defined as the proportion of serum samples with a titer of > 1:500. Prevalence of IgG anti-SE50A was defined as the proportion of serum samples with OD greater that the mean OD of the naïve European controls plus 2 standard deviations.

Hemoglobin electrophoresis. Patient blood was centrifuged and the red cell pellets washed in saline and stored at −20°C until ready for use. Hemolyzed patient samples and the hemoglobin controls (HbAA (normal adult blood), HbAS (blood from a subject with the sickle cell trait), HbAF (blood from an infant below 3 months), and HbAC (blood from a person with an HbAc trait)) supplied by Helena Biosciences (Sunderland, UK) were resolved by electrophoresis on cellu-
lose acetate plates at 350 V for 25 minutes in alkaline buffer (pH 8.4–8.6) and stained with Ponceau S for 5 minutes. The plates were de-stained, dried, and stored for permanent record keeping. The hemoglobin types were identified by comparing the migration of bands in the unknown specimens with the hemoglobin controls.

RESULTS

Summary of clinical and demographic data. The median age and weight, as well as history of antimalarial drug and mosquito bed net use, female/male ratio, and the prevalence of the HBAS genotype were comparable between SM and UM children (Table 1). The commonest symptoms observed in SM children in Apac hospital were respiratory distress (35.4%) and prostration (40.4%) followed by circulatory collapse (7.4%), severe anemia (HB < 5 g/dL, 7.0%), and seizures (2.6%). Mild anemia (HB < 7 g/dL) in SM and UM children were comparable (32.4% versus 22.8%, P = 0.166). None of the SM children had impaired consciousness, coma, or cerebral malaria. Among the UM children, there were 19.6% and 80.4% mild and asymptomatic malaria, respectively.

SM children had significantly elevated measures of morbidity by comparison with UM children, namely higher percent and median numbers of pigmented WBC and temperature. By contrast, UM children had a significantly higher median PCV than SM children but comparable hemoglobin levels by comparison with UM children. When the PCV and hemoglobin levels were compared across three age groups in SM children, there was a distinct age effect. The median and interquartile range (IQR) of PCVs in children 24, 24, and 25 months old were comparable (Mann-Whitney U = 1,008.5, P = 0.0449 and 497.5, P = 0.0171), respectively. The median (and IQR) of hemoglobin levels in SM children 24, 24, and 25–59 months old were comparable (Mann-Whitney U = 301, P = 0.3182), they were significantly greater than those in SM children 6–12 months old (Mann-Whitney U = 1,008.5, P = 0.0449 and 497.5, P = 0.0171), respectively. The median (and IQR) of several measures of morbidity between the two subcategories of UM children, namely those with mild (temperature ≥ 37.5°C and parasitemia ≤ 5,000 parasites/µL; N = 19) and asymptomatic (temperature 37.0°C and parasitemia ≤ 5,000 parasites/µL; N = 78) malaria. With the exception of median temperature (38.0 [0.65] versus 36.3 [0.93]; Mann-Whitney U = 1,463; P < 0.0001), there were no significant differences between median parasitemia density (1,240 [2,680] versus 1,400 [2,000]; Mann-Whitney U = 484; P = 0.70), hemoglobin (8.0 [2.6] versus 8.1 [2.6]; Mann-Whitney U = 729; P = 0.98), packed cell volume (24.6 [6.5] versus 27.4 [9.8]; Mann-Whitney U = 622.5; P = 0.61), and pigmented WBC (0.0 [2.0] versus 0.0 [1.5]; Mann-Whitney U = 638; P = 0.99) in mild and asymptomatic ma-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SM (N = 103)</th>
<th>UM (N = 102)</th>
<th>Statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>13.0 (12)</td>
<td>13 (13)</td>
<td>Mann-Whitney U 5,077</td>
<td>0.9588</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>8.4 (2.7)</td>
<td>8.1 (2.9)</td>
<td>Mann-Whitney U 4,609</td>
<td>0.8119</td>
</tr>
<tr>
<td>Female/male ratio</td>
<td>55/47</td>
<td>50/51</td>
<td>Chi-square test 0.24</td>
<td>0.6247</td>
</tr>
<tr>
<td>Drug use (%)</td>
<td>70.5</td>
<td>76.3</td>
<td>Chi-square test 0.55</td>
<td>0.4590</td>
</tr>
<tr>
<td>Mosquito net use (%)</td>
<td>38.2</td>
<td>43.5</td>
<td>Chi-square test 0.40</td>
<td>0.5291</td>
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<tr>
<td>Pigmented WBC (%)†</td>
<td>0.4 (1.6)</td>
<td>0 (0.2)</td>
<td>Mann-Whitney U 7,249</td>
<td>&lt;0.0001</td>
</tr>
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<td>Pigmented WBC</td>
<td>3.0 (9.0)</td>
<td>0 (1.3)</td>
<td>Mann-Whitney U 6,039</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pigmented WBC (%)‡</td>
<td>81.0</td>
<td>35.7</td>
<td>Chi-square test 34.6</td>
<td>&lt;0.0001</td>
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<tr>
<td>Packed cell volume</td>
<td>23.3 (7.8)</td>
<td>26.7 (8.8)</td>
<td>Mann-Whitney U 3,653</td>
<td>0.0097</td>
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<td>Hemoglobin (g/dL)</td>
<td>7.7 (3.4)</td>
<td>8.1 (3.0)</td>
<td>Mann-Whitney U 4,470</td>
<td>0.1034</td>
</tr>
<tr>
<td>Temperature</td>
<td>38.5 (1.3)</td>
<td>36.7 (1.1)</td>
<td>Mann-Whitney U 8,465</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Parasite density</td>
<td>29,100 (38,780)</td>
<td>1,520 (2400)</td>
<td>Mann-Whitney U 7,909</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HBAS (%)</td>
<td>6.0</td>
<td>11.0</td>
<td>Chi-square test 2.19</td>
<td>0.3348</td>
</tr>
</tbody>
</table>

SM, severe and complicated malaria; UM, uncomplicated or mild malaria; WBC, white blood cells. Significant P values are in bold.
† The values are median (IQR) or prevalence (%).
‡ Percentage of pigmented WBC counted.
‡ Percentage of children with slides positive for pigmented WBC.
P. falciparum

Specific total IgG antibodies against recombinant constructs of SERA5 and P. falciparum schizont lysate. The level of total IgG antibodies against recombinant constructs of SERA5 and a total P. falciparum schizont lysate is shown in Table 3. The prevalence and median titer of IgG anti-SE36 antibodies in UM children were significantly higher than those in SM children (P = 0.0012 and < 0.0001, respectively). The range of antibody titers in individual children was 1:500–1:32,000 in both SM and UM children. To establish whether the higher antibody levels in UM children was maintained in the different age groups, both SM and UM children were subdivided into the age groups 6–12, 13–24, and 25–59 months (Figure 1). The median (IQR) titers of IgG anti-SE36 antibodies in UM children in the 6–12 and 13–24 months old group were significantly higher than those in SM children of the same age groups (2,000 [3,000] versus 500 [750]; Mann-Whitney U = 569, P < 0.0001 and 3,000 [3,000] versus 1,000 [1,500]; Mann-Whitney U = 238, P = 0.0002, respectively). However, the median (IQR) titers of IgG anti-SE36 in UM and SM children in the 25–59 months age groups were not significantly different (2,000 [3,000] versus 1,000 [1,000]; Mann-Whitney U = 94, P = 0.4686). Thus UM children maintained significantly higher titers of IgG anti-SE36 antibodies up to the age of 24 months, whereas in the SM children, there was an age-dependent increase in titers of IgG anti-SE36 (Pearson’s r = 0.21; P = 0.0484), and the older 25–59 months old SM children had a significantly higher median (IQR) titer of IgG anti-SE36 antibodies than the 6–12 months old SM children (1,000 [1,000], N = 17 versus 500 [750], N = 50; Mann-Whitney U = 582, P = 0.0168).

The ODs of IgG anti-SE50 antibodies were generally low in both groups of children, ranging from 0.07 to 0.454 (5.0% to 32.2% of the mean OD for adult positive control sera) and 0.069 to 1.492 (5.0% to 94.5% of the mean OD for adult positive control sera) in SM and UM children, respectively. However, the prevalence and median ODs of IgG anti-SE50 antibodies in both SM and UM children were comparable (P = 0.6990 and 0.2514, respectively; Table 3). As for IgG anti-SE50, the ODs for IgG anti-P. falciparum lysate antibodies were low and ranged from 0.02 to 1.534 (0.4% to 84% of the mean OD for adult positive control sera) in SM children and 0.03 to 2.263 (1.7% to 95.2% of the mean OD for adult positive control sera) for UM children. The prevalence of IgG anti-P. falciparum lysate antibodies in SM and UM children were 73.7% and 94.1%, respectively (Table 3). Thus, approximately 4 out of 5 SM children appeared to have been exposed to P. falciparum infection as judged by the prevalence of IgG anti-P. falciparum lysate antibodies.

High titers of IgG anti-SE36 antibodies are associated with protection against severe malaria. To confirm the hypothesis that higher titers of IgG anti-SE36 antibodies are associated with protection against some of the manifestations of severe malaria, both SM and UM children were classified into two groups on the basis of titers of IgG anti-SE36 antibodies: children with low titers (1:500–1:1000) and children with high titers (> 1000) as shown in Table 2. In SM children, the median ages of children with low versus high titers were comparable (P = 0.4500). However, measures of morbidity such as median temperature and parasite density and % pigmented WBC were reduced in SM children with high titers of IgG anti-SE36 antibodies by comparison with those in SM children with low titers; this difference was highly significant for pigmented WBC (P = 0.0003). Furthermore, median hemoglobin and PCV levels were significantly higher in SM children with high titers of IgG anti-SE36 antibodies than in SM children with low titers (P = 0.003 and 0.0420, respectively). Although respiratory distress and prostration were the commonest manifestations of severe malaria, there was no significant association between these clinical presentations and titers of IgG anti-SE36 antibodies in SM children (prostration, P = 0.645; and respiratory distress, P = 0.887). In UM children, the median ages of subjects with low versus high titers were comparable (P = 0.7910) and, with the exception of temperature, the parasite density, hemoglobin, PCV, and % pigmented WBC were also comparable in both groups of UM children.

The foregoing results suggest that high IgG anti-SE36 titers might be associated with a protective effect against certain measures of morbidity especially in SM children. The relationship between titers of IgG anti-SE36 and protection was therefore further investigated. First, correlation analyses demonstrated that in SM children, titers of IgG anti-SE36

### Table 2

Malaria-related parameters in Ugandan children as a function of IgG anti-SE36 titers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IgG anti-SE36 titer</th>
<th>Mann-Whitney U</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500–1000</td>
<td>&gt; 1000</td>
<td></td>
</tr>
<tr>
<td>Severe malaria SM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>12.0 (11.0)</td>
<td>15.5 (11.0)</td>
<td>1,224.5</td>
</tr>
<tr>
<td>Temperature</td>
<td>38.7 (1.1)</td>
<td>38.1 (2.0)</td>
<td>863.0</td>
</tr>
<tr>
<td>Parasite density</td>
<td>36,000.0 (43,480)</td>
<td>19,880.0 (42,880)</td>
<td>638.0</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>7.3 (2.9)</td>
<td>8.9 (3.3)</td>
<td>1,519.0</td>
</tr>
<tr>
<td>Packed cell volume</td>
<td>22.6 (6.6)</td>
<td>26.7 (9.5)</td>
<td>1,266.0</td>
</tr>
<tr>
<td>Pigmented WBC (%)</td>
<td>0.6 (1.8)</td>
<td>0.0 (0.4)</td>
<td>626.5</td>
</tr>
<tr>
<td>Uncomplicated malaria (UM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>12.5 (15.3)</td>
<td>13.0 (12.0)</td>
<td>854.0</td>
</tr>
<tr>
<td>Temperature</td>
<td>37.0 (1.4)</td>
<td>36.4 (1.0)</td>
<td>574.0</td>
</tr>
<tr>
<td>Parasite density</td>
<td>1,600 (2,340)</td>
<td>1,200.0 (2,280)</td>
<td>577.0</td>
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<tr>
<td>Hemoglobin</td>
<td>8.1 (2.3)</td>
<td>8.5 (3.3)</td>
<td>853.5</td>
</tr>
<tr>
<td>Packed cell volume</td>
<td>28.0 (9.3)</td>
<td>25.2 (8.7)</td>
<td>761.5</td>
</tr>
<tr>
<td>Pigmented WBC (%)</td>
<td>0.0 (0.1)</td>
<td>0.0 (0.2)</td>
<td>969.5</td>
</tr>
</tbody>
</table>

WBC, white blood cells. Significant P values are shown in bold. The sample size for the antibody titer groups 500–1000 and > 1000 were 34 and 52 for SM and 70 and 32 for UM children, respectively.

* The values are median (IQR) or prevalence (%).
antibodies were positively correlated with levels of PCV (Pearson’s r = 0.24, P = 0.0163; N = 96) and hemoglobin (Pearson’s r = 0.39, P < 0.0001; N = 101) and inversely correlated with parasite density (Pearson’s r = −0.23, P = 0.0314; N = 90) and numbers of pigmented leukocytes (Pearson’s r = −0.32, P = 0.0012; N = 101). None of these correlations were significant in UM children. Second, a linear logistic regression analysis (Intercooled Stata for Windows, version 8.2, Stata Corp., 2004), was carried out and after adjusting for the effect of age, sex, and bed net use, the odds ratio (OR) of being in the SM group per unit increase in antibody titer was still significant (OR 0.0370, standard error 0.0289; z = −4.30; P = 0.0001). These data suggest that IgG anti-SE36 antibodies are associated with protection against severe malaria and, significantly, that this protection is independent of age and other possible confounders.

**DISCUSSION**

The manifestations of severe malaria in subjects resident in malaria-endemic areas are not only protean but vary in different geographic settings or endemic regions. In the current study at Apac Hospital in Northern Uganda with a limited number of SM children, the two commonest manifestations were respiratory distress and prostration. Other manifestations such as seizures, severe anemia, and circulatory collapse were observed but were not common (2.6–7.0%). The paucity of severe anemia was surprising in a region with very intense malaria transmission. However, a similar paucity of severe malaria anemia (1.5–2.5%) has been reported in other sites in East Africa. Mild anemia was observed in both SM and UM children and was probably due to other causes that were not fully investigated including hookworm infestations and nutritional iron-deficiency. Interestingly, none of the SM children had impaired consciousness, coma, or cerebral malaria, which are hallmarks of severe malaria in other regions. These findings underscore the importance of cataloging the manifestations of severe malaria in different endemic regions to implement the appropriate adjunct treatment regimens to reduce or preempt morbidity and mortality.

There is evidence that the serine-rich antigen is a promising asexual blood stage vaccine candidate. Our previous work provided some, albeit indirect, evidence that humoral responses against the amino terminal part of SERA5 appeared to be protective in older Ugandan children and adults. However, there is a paucity of immuno-epidemiologic studies of SERA5 in African children under 5 years old who are resident in malaria-endemic regions. This is the first detailed study of natural antibody responses to a recombinant construct of SERA5 in Ugandan children belonging to this age group. Our study provided evidence that high titers of total IgG antibodies against SE36, a recombinant construct of SERA5, were associated with protection against some of the manifestations of severe malaria. UM children had significantly higher median titers of IgG-anti-SE36 than age-matched SM children. It is unlikely that passive immunity passed on by immune mothers during breast-feeding accounted for the higher median titers of IgG-anti-SE36 in children 25–59 months old in both SM and UM children because children are weaned by 2 years of age in this community. The differences in titers of IgG anti-SE36 antibodies in SM and UM children might be attributed to differences in their exposures to *P. falciparum*. Absorption of antibodies by the higher biomass of parasites in the SM group, absorption of antibodies by the higher biomass of parasites in the SM group, and differences in the prevalence of the sickle cell trait, which is protective against severe malaria, and differences in the prevalence of HIV-1 infection. These explanations were excluded for the following reasons. First, the children resided in the same sub-

**FIGURE 1** Median titers of IgG anti-SE36 antibodies as a function of age in 6–12, 13–24, and 25–60 months old Ugandan children with uncomplicated (UM, shaded bars) and severe and complicated (SM, open bars) *falciparum* malaria. The interquartile range (IQR) values are indicated in the text. The statistical significance of the differences between UM and SM in the various age groups are as follows: *P < 0.0001 in the 6–12 month old children; **P = 0.0002 in the 13–24 month old children; ***P = 0.4686 in the 25–60 month old children.
County and were therefore equally at risk of *P. falciparum* infection, and a Gambian study failed to find an association between disease severity and exposure in children.\(^3\) This was confirmed by the fact that about 3 out of 4 SM children had IgG antibodies against total *P. falciparum* schizont lysate. Second, both SM and UM children had comparable mean optical densities (ODs) for total IgG anti-SE50A, a recombinant construct representing the central domain of SERA5 with homology to cysteine proteases.\(^24\) Third, the prevalence of the protective HBAs genotype was comparable in both SM and UM children. Fourth, although we did not investigate the prevalence of HIV-1 serostatus in SM and UM children, we rigorously excluded children with clinical evidence of any immunodeficiency and opportunistic infections suggestive of HIV-1/AIDS.

Further evidence for the association between titers of IgG anti-SE36 and protection against severe malaria was established. First, SM children with higher median titers of IgG-anti-SE36 had significantly higher median hemoglobin and packed cell volumes than SM children of comparable age with lower median titers. Furthermore, SM children with higher median titers of IgG-anti-SE36 were less febrile and had a lower median parasite density; these differences were not statistically significant probably due to the lower sample sizes of the antibody subgroups. Second, there was an inverse correlation between titers of IgG-anti-SE36 and measures of morbidity such as pigmented WBC and parasite density in SM children. Finally, after adjusting for the effect of confounding variables such as age, sex, and bed net use in a linear logistic regression analysis, the OR of being in the SM group per unit increase in antibody titer was significantly reduced.

The different measurements of pigmented WBC (prevalence of slides with pigmented WBC, % pigmented WBC, and numbers of pigmented WBC per 500 WBC counted) were all significantly different between SM and UM children. In addition, our study showed that measurements of pigmented WBC in SM children with high and low antibody titers against SE36 antigen were significantly different. Previous studies have documented the usefulness of pigmented WBC as an index of malaria disease severity in African children and Thai adults.\(^25\)–\(^29\) Our studies have confirmed and extended these findings to demonstrate that measurements of pigmented WBC are inversely correlated with putative protective immunity. The usefulness of measurements of pigmented WBC as an end point in the evaluation of the protective efficacy of malaria vaccines in children remains to be investigated in further studies.

In summary, the current immuno-epidemiologic study of severe malaria in Ugandan children resident in Apac District clearly suggest that higher titers of IgG-anti-SE36 are associated with protection against severe malaria. It is imperative that these studies are repeated in other endemic regions to confirm the association between serum levels of antibodies against SE36 and protection. This should pave the way for Phase I–II clinical trials of malaria vaccines based on SE36 in African children resident in malaria-endemic regions.

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