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Immunoglobulin G Isotype Responses to Erythrocyte Surface-Expressed Variant Antigens of *Plasmodium falciparum* Predict Protection from Malaria in African Children

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We assessed immunoglobulin G (IgG) isotype responses to variant surface antigens (VSA) expressed on parasite-infected erythrocytes of a panel of heterologous isolates during and after acute episodes in groups of Gabonese children presenting with either mild or severe *Plasmodium falciparum* malaria. In the acute and convalescent phases IgG3 and IgG1 anti-VSA antibodies, respectively, predominated. In the absence of infection, the levels of both cytophilic isotypes waned, while those of IgG4 increased, particularly in those admitted with severe malaria. Prospective analyses showed significantly longer delays between malaria attacks associated both (i) with increasing IgG1 responses with specificity for VSA of isolates from children with mild malaria and (ii) with increasing IgG4 responses with specificity for VSA of isolates from children with severe malaria. These findings imply that the predictive value of prospectively measured cross-reactive VSA-specific IgG antibodies with respect to protection against malaria in African children depends both on their isotype and on their fine specificity.

A mounting body of evidence supports the idea that antibody responses directed to *Plasmodium falciparum* variant surface antigens (VSA) inserted into the surface membranes of infected erythrocytes (iE) contribute to the acquired immune protection against malaria caused by this protozoan parasite (2, 9, 13, 26, 36). The VSA described to date include *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1) (33) and the rifins (1, 10, 22). Adhesion of iE to vascular endothelial receptors via these VSA is thought to play a role in the pathogenesis of malaria (8, 27). Anti-VSA antibodies may serve to prevent these adherent interactions, thereby leading to removal of iE in the spleen, and/or to opsonize iE for uptake by phagocytes (14, 37). Such antibody-based protective mechanisms form the basis of a cumulative-exposure model in which the acquisition and maturation of these responses over time leads to the establishment of an antibody repertoire with broad specificity covering the range of VSA expressed by a given parasite population (15). Refinements of this model based on the profiles of antibody-mediated recognition of VSA expressed by diverse isolates suggest the existence of putative rare and common variants associated with mild and severe malaria, respectively (3, 4, 28).

Opsonization of iE presupposes the generation of cytophilic immunoglobulin G (IgG) antibody isotypes in the anti-VSA antibody repertoire, but there are few published data concerning this topic. IgG1 antibodies predominate in the responses of semi-immune Papua New Guinean adults to the VSA expressed by heterologous parasite isolates, in contrast to the profile observed in Gabonese adults, in which IgG3 is predominant (6, 31). We were therefore interested to know whether African children exposed to intense and perennial transmission of *P. falciparum* exhibit a similar isotypic profile of anti-VSA IgG antibodies. Data from a small-scale Kenyan study have, in addition, suggested that children who are susceptible to severe malaria may display altered dynamics of anti-VSA antibody responses, which is in accord with our own recent report (5, 36). Here we addressed this question further through comparison of the IgG isotype profiles of anti-VSA antibodies in Gabonese children with differing outcomes of infection in terms of the clinical severity of *P. falciparum* malaria. For this purpose we used flow cytometric techniques with plasma samples taken at different times either during or after a malaria episode in a cohort of age- and gender-matched Gabonese children who presented with either mild or severe malaria in order to assess changes in the profiles of IgG isotype antibodies directed to the VSA expressed by a panel of six (two putatively rare and four common) heterologous *P. falciparum* isolates. Our own published work has indicated differences in susceptibility to *P. falciparum* infection in terms of both significantly shorter delays to the first reinfections and significantly higher annual malaria attack rates in the group of children who presented with severe rather than mild malaria in this study (23, 24). We therefore also sought associations between these particular parameters and appropriate prospective measures of the children's immune responses, here represented by their convalescent-phase anti-VSA IgG antibody isotype activity.

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MATERIALS AND METHODS

Study site. The study was conducted at the Albert Schweitzer Hospital in Lambaréné, Gabon. The hospital is situated in an area where malaria is hyperendemic and caused predominantly by *P. falciparum* and where transmission is perennial, with an estimated annual entomological inoculation rate of ~50 (34, 39).

Ethical clearance. Ethical clearance for the study was obtained from the ethics committee of the International Foundation of the Albert Schweitzer Hospital in Lambaréné. Informed consent for inclusion into the study was obtained from the parents or guardians of each participating child.

Study design. The study population comprised a subgroup within a matched-pair cohort study of 200 Gabonese children, half of whom presented with severe malaria and half of whom presented with mild malaria due to *P. falciparum*. Inclusion into the study occurred in the period 1995 to 1997. For the purposes of the assessments described here, a subgroup of 60 children, i.e., 30 matched pairs, was selected on the basis of the availability of plasma samples from these individuals at all three study time points (see below). These children's ages ranged from 13 to 101 months, with a mean of 52 months. Severe malaria cases were matched to mild malaria controls according to their age, gender, and provenance. Severe malaria was defined as severe anemia (hemoglobin of <50 g/liter) and/or hyperparasitemia (>250,000 parasites/μl, corresponding to >10% infected erythrocytes) with or without other signs of severe malaria. Samples from children with severe malaria who received blood transfusions were not included. Mild malaria was defined as a parasitemia of between 1,000 and 50,000 parasites/μl of blood, hemoglobin of >8 g/dl, glycemia of >50 mg/dl, and no signs of severe malaria. Children were excluded if they had either concurrent acute infection, previous hospitalization for malaria, intake of antimalarials during the week preceding admission, or any chronic diseases or malnutrition. Details of patient care and treatment have been given elsewhere (20, 21).

Plasma samples. The plasma samples used here were isolated from undiluted venous blood taken on three separate occasions: (i) on the day of admission to the hospital, just prior to administration of antimalarial chemotherapy (here referred to as the acute-phase sample); (ii) 1 month posttreatment (referred to as the convalescent-phase sample); and (iii) at least 6 months posttreatment, when the children had no clinically obvious infection and had been aparasitemic for the preceding 6 weeks, as determined during the active surveillance at 2-week intervals undertaken in the participants' homes following discharge from the hospital (here referred to as the healthy-phase sample). The active in-home surveillance referred to above allowed identification of reinfections (new infections or malaria episodes) through examination of routinely prepared and Giemsa-stained thick blood smears. Children diagnosed with malaria (defined as any *P. falciparum* parasitemia with a rectal temperature of >38°C or clinical symptoms) during this follow-up period were given standard antimalarial treatment with sulfadoxine-pyrimethamine. The time to first reinfection was defined as the time from admission until the time when the first thick blood smear containing parasites was detected.

Plasma samples from 30 nonimmune German adults and from 21 semi-immune Gabonese adults resident in Lambaréné were included as negative and positive controls, respectively.

Parasite isolates and culture. Six *P. falciparum* isolates collected from Gabonese children recruited in a separate outpatient study carried out during 1997 at the Albert Schweitzer Hospital were used. The reference isolates designated Cys002, Cys007, Cys030, and Cys035 (here referred to as VSA_{SM}) were obtained from children with severe *P. falciparum* malaria, and Cym030 and Cym033 (VSA_{UM}) were obtained from children with mild *P. falciparum* malaria. All isolates were confirmed microscopically as monoinfections with *P. falciparum*, and all were shown by routine standardized merozoite surface protein-based PCR genotyping techniques to be polyclonal, each with at least three different strains (C. Yone, unpublished observations). Details of the methods used for collection and culture of parasites have been given elsewhere (35). Briefly, peripheral venous blood was centrifuged, and the erythrocytes obtained were spin washed twice. Pellets containing infected erythrocytes were then cryopreserved in liquid nitrogen for subsequent in vitro adaptation.

Primary isolates were subsequently adapted to in vitro culture according to the method of Trager and Jensen (38). Briefly, cells were resuspended in complete medium supplemented with 10% heat-treated, prescreened, nonimmune AB+ serum (from the blood bank of the University Hospital, Tübingen, Germany), and were then incubated in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. Fresh O+ erythrocytes depleted of lymphocytes (University Hospital, Tübingen, Germany) were periodically added. Isolates were initially expanded over a short period of 8 to 10 48-h multiplication cycles, after which identical stabilates of cultures containing mostly asexual ring forms were cryopreserved for later use in cytometric assays (see below).

Flow cytometric measurement of *P. falciparum*-infected erythrocyte surface-specific IgG isotype responses. Detection of IgG with specificity for the surface of *P. falciparum* trophozoite-infected erythrocytes was performed by using a flow cytometric assay described in detail elsewhere (31, 35). Briefly, iE were enriched by flotation on Plasmagel (Fresenius, Louviers, France) and were then tested for their capacity for binding to the endothelial receptor CD36 expressed on an amelanotic melanoma cell line (C32MC). Binding of iE of each isolate was shown to be maintained at a consistently high level, indicating no loss of the cytoadherent phenotype. iE were then sequentially incubated for 30 min at room temperature with test or control plasma samples diluted 1:50 in phosphate-buffered saline (PBS)–1% bovine serum albumin (BSA), followed by mouse anti-human IgG1, IgG2, IgG3, or IgG4 monoclonal antibody (SkyBio Limited, Wyboston, Bedford, United Kingdom) diluted 1:50 in PBS–1% BSA and then with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Southern Biotechnology, Birmingham, Ala.) diluted 1:100 in PBS–1% BSA and containing 0.5 μg of ethidium bromide per ml. Cells were spin washed twice with PBS–1% BSA after each incubation. Finally, iE were resuspended in PBS and analyzed on a FACScan flow cytometer with CellQuest software (Becton Dickinson, Heidelberg, Germany).

Sample and data analysis. Samples were segregated on the flow cytometer into iE and uninfected erythrocytes (uE) by using forward- and side-scatter parameters, and a gate defining fluorescing (ethidium bromide-stained) cells further segregated parasite-infected cells. Counting 10,000 events per sample and using the geometric mean of the emitted fluorescence intensity (MFI), the amounts of individual IgG isotypes specifically bound to the surface of iE were estimated by application of the formula MFI = (MFI iE test − MFI uE test) − (MFI iE NIP − MFI uE NIP), where NIP represents a pool of nonimmune (German) plasma samples. A threshold value of positivity was established for each IgG isotype and isolate by using the panel of plasma samples from nonexposed Germans, such that test samples were considered anti-VSA IgG isotype responders when the MFI calculated with the equation above was greater than the mean plus two standard deviations of the values obtained with these control samples.

Statistical methods. Data were analyzed by using the Statview and STATA software programs. For paired and unpaired comparisons of continuous variables, the nonparametric Wilcoxon sign rank and Kruskal-Wallis or Mann-Whitney U-test were used, respectively. Contingency tables with continuity corrections were used to compare proportions within and between groups. Correlations between continuous variables were assessed with the nonparametric Spearman rank test corrected for ties, where a rho value of >0.25, concomitant with a P value of <0.05, was considered significant. Survival analyses, using the Cox proportional hazards model, were used to analyze the relationship between IgG isotype anti-VSA antibodies and time to first posttreatment reinfection. MFI values for each IgG isotype for all isolates or separately for VSA_{SM}- or VSA_{UM}-specific antibodies were entered into this model. For this purpose, 49 reinfections recorded in 57 subjects were included. The Cox proportional hazards model with multiple failure events was used to analyze the relationship between IgG isotype anti-VSA antibodies and the delay between reinfections in each individual. For this, a recorded total of 381 reinfections during a total follow-up of 258 years (mean incidence, 1.5 infections per person per year) was included. In both cases, clinical status at admission (severe or mild malaria) was included in the model as a confounding variable. The level of statistical significance in all cases was set at a P value of <0.05.

RESULTS

Quantitative comparison of IgG isotype responses to heterologous *P. falciparum* VSA: within- and between-group comparisons. In order to compare the anti-VSA antibody responses within and between the two groups of children with differing clinical presentation at admission, the MFIs of individual IgG isotype responses to the two VSA_{UM} heterologous parasite isolates and to the four VSA_{SM} were separately pooled for each child (Fig. 1). In acute-phase responses of both groups, VSA_{SM}-specific IgG3 antibodies were the highest, while VSA_{SM}-specific IgG2 responses at this time were significantly higher in the severe malaria group than in the mild malaria group (Fig. 1B and C). In the convalescent phase the

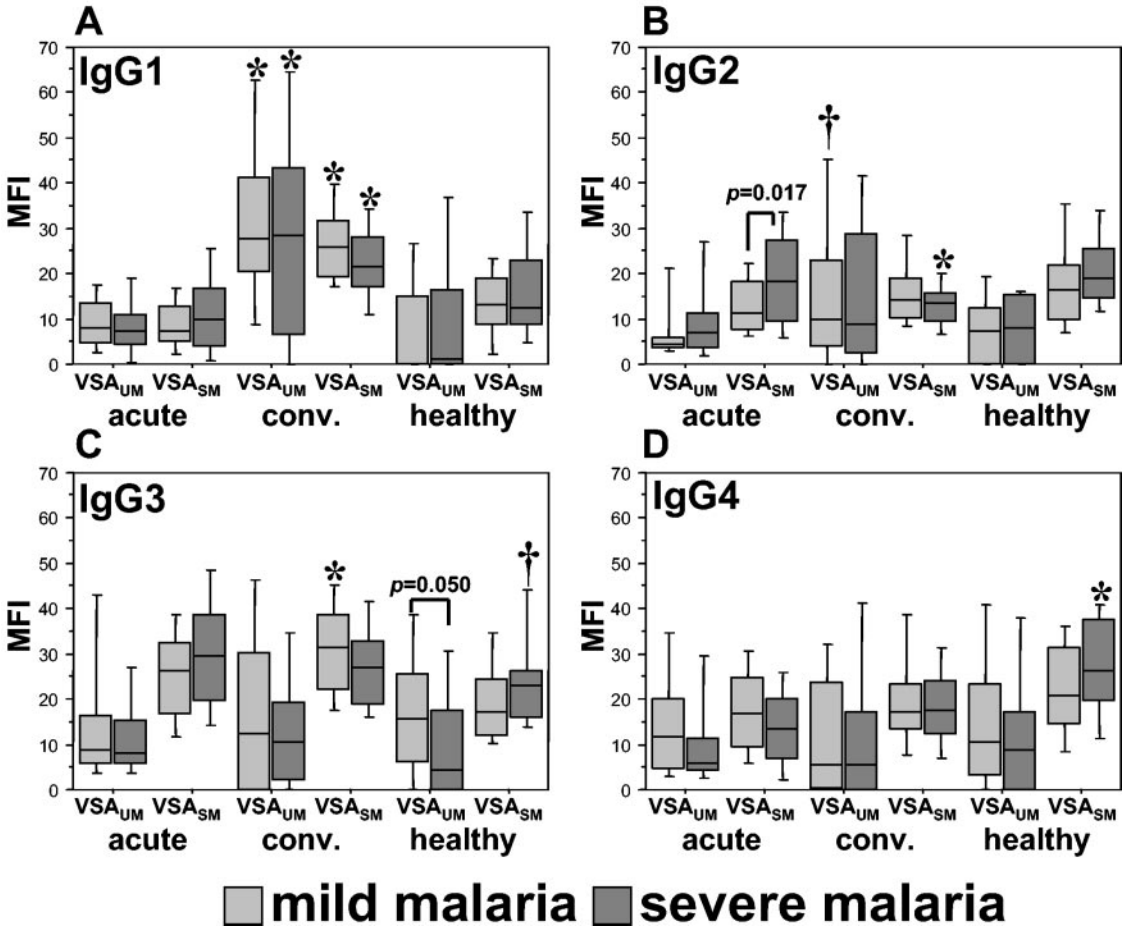

FIG. 1. Magnitudes of IgG isotype responses to heterologous *P. falciparum* VSA. Temporal changes in VSA-specific IgG1 (A), IgG2 (B), IgG3 (C), and IgG4 (D) responses, calculated for each individual as the pooled VSA_{UM}-specific or VSA_{SM}-specific MFI from assays with six heterologous *P. falciparum* isolates, in the acute, convalescent (conv.), and healthy phases and segregated according to children's clinical presentation at admission are shown. Box-whisker plots represent medians with 25th and 75th percentiles and whiskers for 10th and 90th percentiles of the mean MFI. *P* values are derived from the Wilcoxon sign rank test for paired comparisons. *, $P < 0.001$ for within-group comparison with both other time points. †, $P < 0.001$ for within-group comparison with acute phase.

VSA_{UM}- and VSA_{SM}-specific IgG1 responses of both groups were significantly higher than the corresponding activities detected in the acute phase (Fig. 1A). At the same time, IgG2 anti-VSA_{UM} and IgG3 anti-VSA_{SM} responses in the mild malaria group increased significantly, but IgG2 anti-VSA_{SM} antibodies in the severe malaria group declined (Fig. 1B and C). In healthy-phase samples of both groups, cytophilic (IgG1 and IgG3) isotype activity of both VSA_{UM} and VSA_{SM} specificities declined, while IgG2 and IgG4 VSA_{SM}-specific responses increased significantly in the severe malaria group (Fig. 1).

Changes in the ratios of cytophilic to noncytophilic isotypes are shown in Table 1. The observed profiles emphasize the relative predominance of cytophilic IgG3 and IgG1 anti-VSA antibodies in the acute and convalescent phases, respectively, in both groups of children and of IgG4 responses in particular in the healthy-phase profile of children in the severe malaria group.

The profiles of convalescent-phase anti-VSA IgG antibody isotype responses with specificity for the individual isolates are illustrated in Fig. 2. No obvious isolate-specific pattern is discernible. For certain isolates e.g., Cym033, Cys030, and Cym030 (Fig. 2C, D, and F), the activity of cytophilic isotypes appears to be relatively greater than that of noncytophilic isotypes, but this pattern did not apply to both groups in all cases; e.g., there was reduced IgG3 activity in the severe malaria group with respect to Cym033 and Cym030 (Fig. 2C and F). No particular isotype-specific predominance was discernible in the profiles of responses with specificity for the VSA_{SM} isolates Cys035 and Cys007 (Fig. 2A and B).

Prospective assessment of associations between convalescent-phase anti-VSA IgG isotype responses and reinfections. Follow-up surveillance showed that children in the severe malaria group had significantly shorter delays to their first post-treatment malaria attack and significantly higher malaria attack rates than their matched counterparts with mild malaria (23, 24). Here, therefore, we performed survival analyses to test the extent of the association between a prospective measure, convalescent-phase anti-VSA IgG antibody isotype responses, and protection from malaria, using Cox's proportional hazards model to determine their influence on either the time

TABLE 1. Temporal changes in the ratios of cytophilic and noncytophilic IgG isotype antibodies with specificity for VSA of heterologous *P. falciparum* isolates in groups of Gabonese children segregated according to the clinical severity of malaria at admission

Ratio	Clinical group	MFI[a] in:			P[b]		
		Acute phase	Convalescent phase	Healthy phase	Acute vs convalescent	Convalescent vs healthy	Acute vs healthy
IgG1/(IgG2 + IgG4)	Mild	0.656 (0.391–1.026)	1.854 (1.425–2.404)	0.780 (0.451–1.165)	<0.001	<0.001	NS[c]
	Severe	0.577 (0.334–1.183)	1.791 (1.075–2.412)	0.661 (0.377–1.180)	<0.001	<0.001	NS
IgG3/(IgG2 + IgG4)	Mild	1.770 (1.13–2.444)	1.393 (1.160–2.343)	1.000 (0.623–1.975)	NS	0.075	NS
	Severe	1.960 (1.22–2.548)	1.517 (1.057–1.922)	0.931 (0.778–1.100)	0.061	0.013	<0.001
IgG2/(IgG1 + IgG3)	Mild	0.463 (0.336–1.104)	0.534 (0.334–0.813)	0.970 (0.543–1.589)	NS	0.010	NS
	Severe	0.918 (0.652–1.174)	0.660 (0.398–0.790)	0.969 (0.640–1.424)	0.005	0.002	NS
IgG4/(IgG1 + IgG3)	Mild	1.040[d] (0.684–1.623)	0.554 (0.428–0.750)	1.090 (0.664–1.921)	0.002	<0.001	NS
	Severe	0.751 (0.420–0.977)	0.527 (0.352–0.873)	1.516 (0.929–1.947)	NS	<0.001	<0.001

[a] Values given are medians (interquartile ranges) of ratios of anti-VSA antibody isotype MFIs.
[b] P values derived from Wilcoxon sign rank test for paired comparisons.
[c] NS, not significant.
[d] $P = 0.017$ (mild versus severe).

to first posttreatment reinfection or the interval between reinfections observed in each child, controlled for clinical presentation status (mild or severe malaria). As expected, reinfection outcomes were found to be significantly influenced by clinical status (Table 2). Consideration of anti-VSA IgG isotype responses without regard for their specificity in this model revealed an independent but nonsignificant trend towards a longer delay to first reinfection with increasing magnitude of IgG1 activity (hazard ratio; 0.968, 95% confidence interval [CI], 0.935 to 1.001; $P = 0.059$) but no such influence for any other IgG isotype (data not shown). Segregation of responses according to their specificity for isolates expressing either VSA_{UM} or VSA_{SM} showed that the trend referred to above was solely attributable to IgG1 antibodies with specificity for VSA_{UM} (hazard ratio 0.987; 95% CI, 0.974 to 1.000; $P = 0.057$), while also revealing separate and independent trends towards associations of IgG2 anti-VSA_{UM} antibodies with longer delays to first reinfection and of IgG3 anti-VSA_{SM} antibodies with shorter delays to first reinfection (hazard ratio, 1.023; 95% CI, 0.997 to 1.049; $P = 0.082$). The results of the

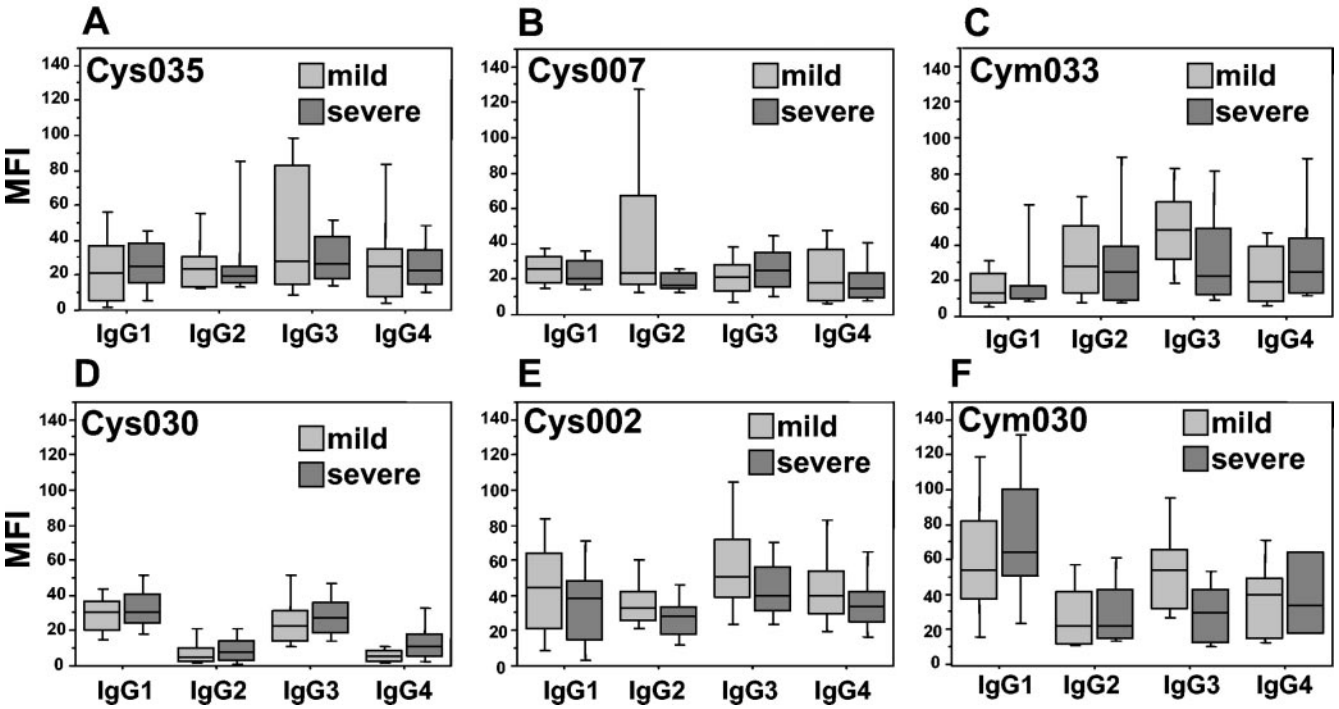

FIG. 2. Profiles of convalescent-phase IgG isotype responses to heterologous *P. falciparum* VSA, segregated according to children's clinical presentation at admission. Box-whisker plots represent medians with 25th and 75th percentiles and whiskers for 10th and 90th percentiles of the mean MFI, excluding nonresponders. Responses to the four VSA_{SM} isolates (A, B, D, and E) are illustrated separately from those to the two VSA_{UM} isolates (C and F).

TABLE 2. Survival analysis, using Cox proportional hazards model with multiple failure events, of the interval between reinfections for convalescent-phase IgG isotype anti-VSA antibody responses

Group[a]	Clinical status or isotype	Hazard ratio	95% CI	*P*
α-VSA_{ALL}	Clinical status	1.306	1.041–1.638	0.021
	IgG1	0.979	0.968–0.991	<0.001
	IgG2	0.998	0.982–1.014	0.807
	IgG3	1.001	0.989–1.013	0.888
	IgG4	0.997	0.983–1.012	0.725
α-VSA_{UM}	Clinical status	1.371	1.105–1.701	0.004
	IgG1	0.990	0.985–0.995	<0.001
	IgG2	0.998	0.991–1.004	0.510
	IgG3	0.996	0.989–1.004	0.316
	IgG4	1.006	0.999–1.012	0.115
α-VSA_{SM}	Clinical status	1.369	1.093–1.713	0.006
	IgG1	0.993	0.981–1.005	0.269
	IgG2	1.000	0.988–1.013	0.999
	IgG3	1.008	0.997–1.019	0.139
	IgG4	0.984	0.973–0.995	0.006

[a] Analyses considered the MFI of anti-VSA responses to all six heterologous isolates together (α-VSA_{ALL}) or after segregation into those with specificity for the two isolates expressing VSA_{UM} or for the four isolates expressing VSA_{SM}.

analyses of the interval between reinfections are shown in Table 2. Here, a highly significant association between longer intervals and IgG1 anti-VSA antibodies was found (hazard ratio, 0.979; 95% CI, 0.968 to 0.991; $P < 0.001$), and this was attributable exclusively to the antibodies with specificity for VSA_{UM} (hazard ratio, 0.990; 95% CI, 0.985 to 0.995; $P < 0.001$) (Table 2). The same analyses revealed a statistically significant association of extended intervals between reinfections and increasing levels of IgG4 anti-VSA_{SM} antibodies (hazard ratio, 0.984; 95% CI, 0.973 to 0.995; $P = 0.006$) (Table 2).

Retrospective assessment of the influence of reinfections on healthy-phase anti-VSA antibody responses. Since the first and subsequent posttreatment reinfections in many individuals occurred in the interval between collection of the convalescent- and healthy-phase samples, we determined the potential influence of reinfections during this period on the profile of healthy-phase anti-VSA antibody IgG isotype activity by assessment of correlations (Spearman rank), using the number of reinfections as a continuous variable, or by direct comparison between groups (Mann-Whitney) following dichotomization according to the presence or absence of reinfection. No statistically significant associations were detected by either test either for the cohort as a whole or when the cohort was segregated according to clinical presentation status with respect to individual IgG isotype activity with specificity for either VSA_{UM} or VSA_{SM} (data not shown).

DISCUSSION

We describe here the evolution of IgG isotype antibody responses to the VSA expressed by heterologous *P. falciparum* isolates as a function both of young African children's clinical presentation at inclusion into the study and of their subsequent reinfection profiles. We specifically excluded samples from children diagnosed with severe malarial anemia who received blood transfusions as part of their supportive treatment in order to avoid the potential confounding effects of passively transferred antibodies in these analyses.

The data are presented as geometric MFIs and are therefore not directly comparable to those of a recently published Kenyan study, which are expressed as the proportion of infected erythrocytes positive for bound antibody (18). The results of the latter study indicated that the IgG isotype response to the VSA expressed by homologous parasite isolates, in children who presented with uncomplicated *P. falciparum* malaria, is composed predominantly, although not exclusively, of IgG3 antibodies. Our data showing that levels of IgG3 anti-VSA antibodies were the highest in samples taken in the acute phase of the infection are consistent with that finding. IgG3 antibodies are the predominant isotype in the profile of responses of healthy semi-immune adult Gabonese, with specificity for the VSA expressed by the same panel of heterologous parasite isolates (6). Where our data diverge from those of the Kenyan study is in the profile of posttreatment (convalescent-phase) anti-VSA responses, in which, in our study, the IgG1 anti-VSA response was clearly predominant (Fig. 1A). This observation is consistent with the results of numerous studies that have reported enhancements of the levels of IgG antibodies with specificity for the VSA expressed by heterologous parasite isolates in the postinfection period (5, 7, 12, 16, 29). Our data thus strongly imply that cross-reactive antibodies are a prominent feature of the profile of anti-VSA responses induced by *P. falciparum* malaria episodes in young African children with high and perennial levels of exposure to infection. This further substantiates our own observations that in some members of the same cohort, IgG1 antibody responses with specificity for VSA of heterologous parasite isolates are also elevated during the first posttreatment malaria episodes that they experienced (6).

The significant decline of the level of IgG3 and enhancement of that of IgG4 anti-VSA responses observed when children were healthy and parasite free are aspects of particular interest in the data we present here. Since IgG3 has the shortest half-life (ca. 8 days) of all of the IgG isotypes, a temporally related decrease in the amount of such antibodies in the absence of parasite antigen-mediated stimulation might be expected. Noncytophilic IgG4 antibodies are reported to interfere with the parasite growth inhibition mediated by cytophilic isotypes in vitro and may therefore act as "blocking" antibodies in vivo (14). Clearly, however, the data we present here indicate that high levels of IgG4 anti-VSA antibodies with a particular VSA_{SM} specificity are beneficial rather than detrimental, in the sense that they are associated with prolonged intervals between malaria attacks. We speculate that they may function by interfering with cytoadherence via blockade of infected erythrocyte-endothelial cell ligand-receptor interactions, but their specificity remains a paradox. IgG4 antibodies are commonly thought to be directed to carbohydrate epitopes, but there is no evidence for carbohydrate epitopes as components of either PfEMP-1 or rifins. Since our own study has shown that IgG4 antibodies represent only a relatively minor component of the anti-VSA response repertoire of healthy semi-immune adults, we conclude that repeated exposure nevertheless results in a change in the clinico-physiopathological relevance of the different IgG isotypes (6). Age-related

switches in IgG isotype activity with specificity for polymorphic determinants have been noted in the profile of at least one other asexual-stage antigen (35).

The particularly outstanding observation of this study concerns the strong association between high convalescent-phase anti-VSA_{UM} IgG1 responses and clinical protection as manifest by significantly prolonged intervals between malaria attacks. This represents persuasive evidence for a protective function of antibodies of the major cytophilic IgG isotype directed to the VSA expressed by heterologous parasite isolates of a particular subtype. Such infection-induced cytophilic antibodies could mediate their effects via targeting of determinants expressed by *P. falciparum* VSA, leading to blockade of infected erythrocyte cytoadherence to endothelial cells, and/or opsonization, leading to phagocytosis through interactions with Fcγ receptors on phagocytic cells (36). The putative principal target of anti-VSA antibodies, PfEMP-1, is known to contain conserved epitopes that are recognized by antibodies from African children and adults (11, 30). Whether these or other epitopes of PfEMP-1, or even of other VSA such as the rifins, are the targets of the protective IgG1 isotype responses that our study has revealed remains to be determined.

Based on coding sequences and chromosomal positional parameters, it has been proposed that PfEMP-1 *var* genes can be segregated into groups encoding variants with greater or lesser degrees of complexity and that the clinical severity of malaria may reflect preferential expression of members of a particular subgroup of these genes (17, 32). We have not determined the precise molecular identity of the PfEMP-1 variants expressed by our panel of isolates, although we do know that they comprise multiple strains (C. Yone, unpublished observations) and also that the donors were young children, with mean ages of 25 and 40 months for the VSA_{UM} and VSA_{SM} donors, respectively. Since putatively rare and common VSA variants are thought to be preferentially expressed in older and younger children, respectively, primarily reflecting differences in the level of acquired immunity (3, 4, 28), we conclude that in the absence of detailed molecular characterization such a distinction cannot be definitively applied to our panel of isolates. Nevertheless, only ~40% of adult Gabonese have IgG1 responses, whereas almost 100% have IgG2 and IgG3 responses with specificity for the two VSA_{UM} isolates and ~75% have IgG4 responses with specificity for the VSA_{SM} isolates of our panel (G. Cabrera, unpublished observations). These observations serve to emphasize both the age-related changes and the apparent differences in the pattern of IgG isotype antibodies induced predominantly by variants expressed by parasite isolates with different origins, differences that presumably lie at the epitope level. Self-evidently, in the study described here the children who presented with severe malaria lacked effective immune responses capable of suppressing the growth of the parasites responsible for their condition. Despite the relatively greater susceptibility to malaria and the relatively poorer persistence of parasite antigen-specific antibodies within this particular group (19, 23–25), the findings we present here nevertheless suggest that an ability to produce larger amounts of anti-VSA antibodies with specificity for determinants expressed by heterologous parasite isolates is associated with a benefit to some of these children in the form of a degree of protection from malaria.

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