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Immunoglobulin M and G Antibody Responses to *Plasmodium falciparum* Glutamate-Rich Protein: Correlation with Clinical Immunity in Gambian Children

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The aims of the present study were to describe the age-related immunoglobulin M (IgM) and IgG response to part of a 220-kDa glutamate-rich protein (GLURP) from *Plasmodium falciparum* and to determine possible correlations of possession of these antibodies with malaria morbidity. IgM and IgG levels were measured with a recombinant fusion protein consisting of the carboxy-terminal 783 amino acids of the GLURP. Samples for the study were obtained during a longitudinal malaria morbidity survey performed in The Gambia; cross-sectional surveys were performed at the beginning of the transmission season in May and in October. Seropositivity rates increased with age to a maximum of 77% for IgM and 95% for IgG in adults. High prevalences of seropositivity were associated with certain human leukocyte antigen class II alleles (*DRw8*, *DR9*, *DR7*, *DR4*, *DQw7*, and *DQw2*) or haplotypes. The relationship between anti-GLURP$_{489–1271}$ antibodies and clinical immunity is not clear; asymptomatically infected children aged 5 to 8 years had significantly higher levels of IgG than clinically ill children of the same age, suggesting that antibodies to the carboxy-terminal part of the GLURP may contribute to immunity to *P. falciparum*. However, this was not significant for younger children.

We have studied the antibody response to a fragment of the 220-kDa glutamate-rich protein (GLURP) from *Plasmodium falciparum*. Immunogold electron microscopy has indicated that the GLURP is present in all stages of the parasite in the vertebrate host, and the gene encoding GLURP is conserved in geographically distant *P. falciparum* isolates (1). A fragment consisting of the carboxy-terminal 783 amino acid residues of the GLURP (GLURP$_{489–1271}$) has been produced as a chimeric fusion protein in *Escherichia coli* and used in an indirect enzyme-linked immunosorbent assay (ELISA) for measurement of immunoglobulin G (IgG) antibodies (4) and in a μ-chain capture ELISA to measure IgM antibodies (5). Previous studies have indicated a high prevalence of anti-GLURP$_{489–1271}$ antibodies in adult immune Liberians (4, 5), and a negative association between IgG response and parasite density has been found in Liberian children (8).

In the present study, a cohort of Gambian children 3 to 8 years old was included in a seroepidemiological survey. The survey started in May, before the malaria transmission season, with an initial determination of antibody status. Malaria morbidity was subsequently surveilled for a period of 6 months. At the end of the transmission season, a cross-sectional survey was carried out with 74 children from the longitudinal study and an extra 166 individuals from ages 2 to 86.

The present paper describes the relation of age to IgM and IgG antibody responses to GLURP$_{489–1271}$ and investigates statistical correlations between possession of anti-GLURP$_{489–1271}$ antibodies and human leukocyte antigen (HLA) class II allele or haplotype and malaria morbidity.

MATERIALS AND METHODS

Study area and subjects. This study was carried out in the Fula and Kataba villages near the town of Farafenni on the north bank of the River Gambia. Transmission of malaria in this region is seasonal, with maximum transmission occurring from May to November. Ninety percent of malaria infections are caused by *P. falciparum*, and 10% are caused by *Plasmodium malariae*. A detailed description of malaria transmission in this area has been published by Greenwood et al. (7) and by Marsh et al. (9).

The population of these villages is composed of approximately equal numbers of three distinct ethnic groups: Fula, Mandinka, and Wollof. The ethnic composition of the cohort of 385 children was as follows: 50% were Fula, 25% were Mandinka, and 25% were Wollof; for the three groups of children and adults studied in October, these figures were as follows: 14% were Fula, 59% were Mandinka, and 28% were Wollof.

All 385 children (age range, 3 to 8 years) participating in the study from May to November went through a health examination in May; venous blood samples were collected, and age and ethnic group were recorded. Blood films were examined for malaria parasites, and parasitemia was treated with chloroquine and Maloprim. From May to November, 350 of the children were visited once a week by a field-

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worker, at which time a health questionnaire was completed and the axillary temperature of each child was measured. A finger prick blood sample to examine malaria parasites was taken from every child who had a temperature of 37.5°C or more. Elevated temperature and parasitemia levels of above 5,000 parasites per μl of blood were recorded as an episode of clinical malaria. Fever and parasitemia levels of <5,000 parasites per μl were recorded as indeterminate. Children in whom parasitemia was detected during the October survey but who had not experienced an episode of fever associated with any parasitemia during the rainy season and children with acquired splenomegaly in the absence of other clinical symptoms and signs were classified as having asymptomatic infections. Acquired splenomegaly was defined as the presence of a palpable spleen in November when there had been no such finding in May or as an increase in spleen size of at least 2 cm during this period. Children in this group were presumed to possess clinical, although not sterile, immunity. An additional group consisted of children with no clinical symptoms and no detectable infection. In November, 74 of these children were retested. An additional 166 individuals aged 2 to 86 years were also bled in November. For full details of the study design, see Riley et al. (13).

Antigen. The antigen used for the assays in the present study was a chimeric β-galactosidase fusion protein containing amino acid residues 489 to 1271 from GLURP. The protein was produced in *E. coli*, purified, and used in an indirect ELISA or biotinylated for use in a μ-chain capture ELISA. Purified β-galactosidase (Boehringer Mannheim) was used in equimolar concentrations as a control for reactivity caused by the β-galactosidase portion of the fusion protein (4).

ELISAs. (i) **IgG.** The assay used for detection of IgG antibodies was an indirect ELISA performed as described previously (4). Briefly, microtiter plates were coated with fusion protein or, for the control, with β-galactosidase. Plasma diluted 1:200 was added to duplicate wells, and the plate was incubated for 1 h at room temperature. Bound antibody was detected with peroxidase-conjugated rabbit anti-human IgG (code P214; Dakopatts, Glostrup, Denmark). To eliminate interassay variation, all measurements were normalized with an optical density at 490 nm (OD_{490}) of 1.200 assigned to the positive control; this value corresponds to the arithmetic mean of 10 independent measurements. The value for the β-galactosidase coating was subtracted, and negative differences were assigned the value zero. The cutoff for a positive result in the assay was determined to be 0.09 OD_{490} units (99th percentile for 105 healthy adult Danish donors).

(ii) **IgM.** The assay for detection of specific IgM antibodies was constructed as a μ-chain capture ELISA with a biotinylated antigen (5). Briefly, microtiter plates were coated with rabbit anti-human μ chain (code A091; Dakopatts). Plasma diluted 1:1,000 was incubated in duplicate wells for 2 h, and specific IgM antibodies were detected by sequential incubation with biotinylated fusion protein for 1 h and peroxidase-conjugated avidin (code P347; Dakopatts) for 1 h. Biotinylated β-galactosidase was run in parallel for each sample, the value for β-galactosidase was subtracted from the fusion protein value, and negative differences were assigned the value zero. A positive control diluted 1:1,000 was included on every plate. To eliminate interassay variation, all measurements were normalized with an OD_{490} of 0.948 assigned to the positive control. This value is the arithmetic mean of 43 measurements performed on individual plates. The cutoff for positive values was determined to be 0.116 OD_{490} units (maximum value obtained with 80 healthy adult Danish donors).

RESA antibodies. Detection of ring-infected erythrocyte surface antigen (RESA) antibodies was carried out as described previously (14).

HLA class II typing. HLA class II (*DR* and *DQ*) typing was performed by Southern blot analysis of *Taq*I-cleaved DNA obtained from peripheral blood leukocytes (2). Allelic restriction fragment length polymorphism patterns at each locus (*DRB*, *DQA*, and *DQB*) were designated by roman numerals (2, 11). Arabic numerals have also been ascribed to individual combinations of *DRB-DQA-DQB* haplotypes.

Statistical methods. Antibody responses to $GLURP_{489-1271}$ were analyzed as $\log_e (OD_{490})$. A χ^2 statistic for the overall effect of HLA polymorphism was calculated from logistic regression. In all cases, the regression allows for the confounding effects of age, sex, and previous malaria control intervention (13). For the May serosurvey, allowance is also made for carriage of sickle cell trait and concurrent parasitemia. The effects of individual haplotypes were evaluated by comparing the ratio of each parameter estimate to its standard error with a normal distribution. Gambian villages are made up of compounds which accommodate large extended families. Thus, in addition to sharing HLA haplotypes and a variable number of genes of non-HLA status in the HLA region, related individuals also share the same environment. The effects of shared background genes and shared environment were removed by stratifying on compound by conditional logistic regression.

RESULTS

Relationship of anti-$GLURP_{489-1271}$ antibodies to age, sex, HLA class II haplotypes, and parasitemia: seroconversion rates. In both the May and the October surveys, the prevalence of anti-$GLURP_{489-1271}$ IgG and IgM seropositivity, i.e., the percentage of individuals with an OD value above the cutoff, increased with age. In October, maxima of 77% seropositivity for IgM for the age group composed of 15- to 24-year-old individuals and 95% seropositivity for IgG for the age group composed of 25- to 44-year-old individuals were reached (Fig. 1). The median level of antibody response for seropositive individuals also increased with age for children and young adults (Fig. 2).

Within individual donors, there was a strong association between anti-$GLURP_{489-1271}$ IgG seropositivity and IgM seropositivity in both the May and the October surveys (χ^2 = 62.1 [$P < 0.0001$] and χ^2 = 37.9 [$P < 0.0001$], respectively). Similarly, there was a high correlation between the magnitude of the $\log_e$ of IgG and IgM responses in both May and October: $r = 0.31$ ($P < 0.0001$) and $r = 0.53$ ($P < 0.0001$), respectively. Analyses of the magnitude of antibody response were based on the median OD value among seropositive individuals or on $\log_e$(OD), since the raw data were highly skewed.

No significant association between seropositivity for IgM and IgG and sex or ethnic group was found in either the May or the October survey (data not shown). However, an analysis of the magnitude of response showed that the median value for IgG in the May survey was greatest for the 66 Fulas (0.579), whereas similar levels were observed for the 24 Mandinkas (0.209) and 34 Wollofs (0.257) (Kruskal-Wallis test, χ^2 = 10.8 and 2 df [$P = 0.005$]). No significant differences for the IgG value in October or for the IgM value in May or October were found. The association between

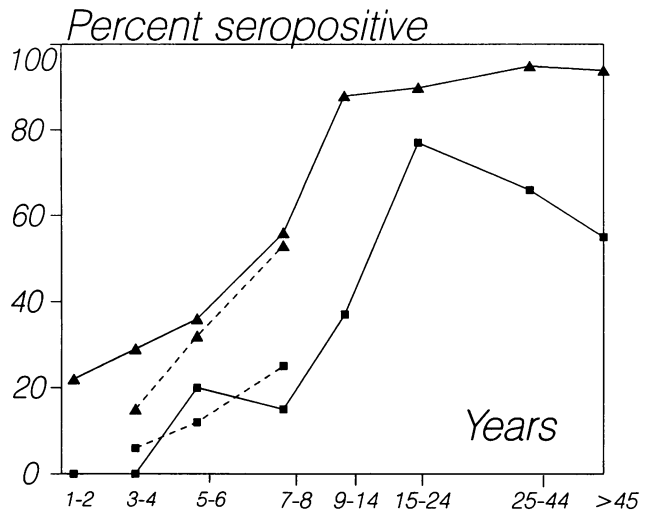

FIG. 1. Relationship of anti-GLURP$_{489-1271}$ seropositivity rate to age. Shown are percentages of seropositive individuals with IgM values above the cutoff ($OD_{490} \geq 0.116$) in May (squares and dotted line) and in October (squares and solid line) and percentages of seropositive individuals with IgG values above the cutoff ($OD_{490} \geq 0.09$) in May (triangles and dotted line) and in October (triangles and solid line).

ethnic group and magnitude of response suggested an effect of genetic background.

All participants were HLA class II typed, and the effect of class II antigen on prevalence of seropositivity was analyzed by using all *DR-DQ* haplotypes ($n = 9$) with a frequency of at least 20 individuals (May) or 10 individuals (October). This analysis was carried out by multiple logistic regression to allow for the confounding effects of sex, age, malaria control interventions, and (in May only) sickle cell trait and presence of parasitemia. Full details of the statistical methods used are described by Riley et al. (15). Overall, there was a significant association between HLA class II haplotype and both IgG and IgM seropositivity in the May survey

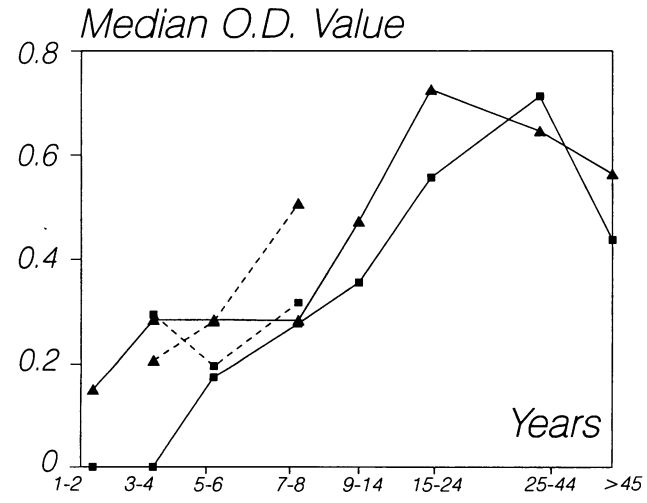

FIG. 2. Relationship of median anti-GLURP$_{489-1271}$ (IgM [squares] and IgG [triangles]) antibody response to age. The median values were calculated for seropositive individuals in each age group for May (dotted lines) and for October (solid lines).

TABLE 1. *DRB*, *DQA*, and *DQB* haplotypes and associated serological specificities[a]

Haplotype	*Taq*I restriction fragment length polymorphism alleles:			Associated serological specificity		Association with[b]:	
	DRB	*DQA*	*DQB*	*DR*	*DQ*	IgG	IgM
7	VIII	IV	III	w17	w2	NS	NS
11	XII	V	IV	4	w8	(*)	NS
12	XII	V	VI	4	w2	**	NS
15	XIV	V	VI	7	w2	*	**
17	V	VI	V	w8	w7	***	NS
21	XVII	V	IX	9	w2	*	NS
22	III	I	I	w10	w5	NS	NS
29	XXI	IV	V	w11	w7	NS	NS
38	X	II	I	w13	w6	NS	NS

[a] Results are from tests for association with seropositivity for anti-GLURP$_{489-1271}$ IgG and IgM in May (11).
[b] *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; (*), $0.05 < P < 0.10$; NS, not significant.

($\chi^2 = 27.8$ and 9 df [$P < 0.01$] and $\chi^2 = 19.7$ and 9 df [$P < 0.02$], respectively). A conditional logistic regression showed that these associations remained significant after the effect of shared environment was taken into account. Individual haplotypes which were significantly associated with higher prevalences of seropositivity of IgG or IgM are listed in Table 1. There was no significant association with either IgG or IgM in the October survey ($\chi^2 = 11.2$ and 3.3, respectively, on 8 df).

Concurrent parasitemia was positively associated with anti-GLURP$_{489-1271}$ IgM seropositivity in both the May and the October surveys ($\chi^2 = 16.7$ [$P < 0.0001$] and $\chi^2 = 3.4$ [$P < 0.07$], respectively) and with IgG seropositivity in the May survey ($\chi^2 = 44.1$ [$P < 0.0001$]) but not in the October survey ($\chi^2 = 1.0$) (Table 2). When age stratified, the associations for IgM and IgG seropositivity in the May survey remained significant (Mantel-Haenszel test (MH), $\chi^2 = 15.1$ [$P < 0.0001$] and $\chi^2 = 43.1$ [$P < 0.0001$], respectively) but that for IgM seropositivity in the October survey became insignificant (MH, $\chi^2 = 1.9$).

The relationship between seropositivity and parasite density was examined by classifying density into the following four categories: no parasites, <500 parasites per μl, ≥500 but <5,000 parasites per μl, and ≥5,000 parasites per μl (Table 3). A test for trend, stratified by age, showed that the proportion of children seropositive for anti-GLURP$_{489-1271}$ IgM and IgG in May increased as parasite density increased ($\chi^2 = 14.0$ [$P < 0.0002$] and $\chi^2 = 43.3$ [$P < 0.0001$], respectively). The number of children with high parasite densities in October was too low to analyze usefully, but no

TABLE 2. Numbers of children with parasitemia according to serological status

Testing period	No. of children with parasitemia/*n* (%)			
	IgM seropositive	IgM seronegative	IgG seropositive	IgG seronegative
May	30/56 (54)[a]	84/329 (26)[a]	65/124 (52)[b]	49/261 (19)[b]
October	9/12 (75)[c]	26/63 (41)[c]	19/35 (54)[d]	16/40 (40)[d]

[a] $\chi^2 = 16.7$ ($P < 0.0001$).
[b] $\chi^2 = 44.1$ ($P < 0.0001$).
[c] $\chi^2 = 3.4$ ($P < 0.07$).
[d] $\chi^2 = 1.0$ ($P > 0.05$).

TABLE 3. Proportion of children seropositive in May according to parasite density and age

Antibody and parasite density[a]	No. of children with antibodies/n (%) in the following age groups:			
	3–4 yr	5–6 yr	7–8 yr	Total
IgM				
No parasites	3/102 (3)	6/91 (7)	17/78 (22)	26/271 (10)
<500	3/19 (16)	7/30 (23)	8/24 (33)	18/73 (25)
500–<5,000	2/12 (17)	6/12 (50)	4/11 (36)	12/35 (34)
≥5,000	0/3	0/2	0/1	0/6 (0)
Total				56/385 (15)
IgG				
No parasites	11/102 (11)	14/91 (15)	34/78 (44)	59/271 (22)
<500	3/19 (16)	20/30 (67)	17/24 (71)	40/73 (55)
500–<5,000	6/12 (50)	7/12 (58)	8/11 (73)	20/35 (57)
≥5,000	1/3 (33)	2/2 (100)	1/1 (100)	4/6 (67)
Total				123/385 (32)

[a] Parasite densities are numbers of parasites per microliter.

correlation between the magnitude of antibody response and parasite density was found (data not shown).

Seroconversion rates during the transmission season among the 74 children included in both the May and the October surveys were estimated. However, these should be interpreted with some caution, since the group was composed of only a subgroup of study children and may not represent the whole population. Of individuals who were negative in May, 7 of 63 (11.1%) became seropositive in October for IgM and 13 of 44 (29.5%) became seropositive in October for IgG. Of individuals who were positive in May, 6 of 11 (54.5%) became seronegative in October for IgM and 8 of 30 (26.7%) became seronegative in October for IgG.

Relationship with RESA antibodies. GLURP is an antigen with a unique amino acid sequence (1); however, its high content of glutamate and the occurrence of glutamate-glutamate dimers raise the issue of serological cross-reactivity with other glutamate-rich proteins of *P. falciparum* (10), e.g., the RESA (3). GLURP might induce antibodies binding to other glutamate-rich antigens in serological tests, e.g., RESA peptide ELISA, and these antigens might induce antibodies binding to $GLURP_{489–1271}$. Therefore, possible associations between measurements of anti-$GLURP_{489–1271}$ antibodies and measurements of anti-RESA antibodies previously performed on the same samples (14) were examined.

TABLE 4. Spearman rank correlation of magnitude of anti-$GLURP_{489–1271}$ IgG response with magnitude of responses to RESA peptides and to EMIF[a] for May and October

RESA peptide or EMIF	r for May (n = 385)[b]	r for October (n = 240)[b]
$EENV_6$	0.303 (<0.0001)	0.427 (<0.0001)
$EENVEHDA_3$	0.223 (<0.0001)	0.387 (<0.0001)
K(DDEHVEEPTVA)	0.206 (<0.0001)	0.386 (<0.0001)
EMIF	0.362 (<0.0001)[c]	0.210 (<0.001)

[a] EMIF, erythrocyte membrane immunofluorescence.
[b] P values are in parentheses. The low P values arise from the large numbers of pairs of data.
[c] n = 382.

TABLE 5. Anti-$GLURP_{489–1271}$ seropositivity in May and subsequent malaria morbidity

Age group (yr)	No. of IgM-seropositive children/n (%) with:		No. of IgG-seropositive children/n (%) with:	
	Clinical malaria	Asymptomatic infection	Clinical malaria	Asymptomatic infection
3–4	6/48 (13)	1/29 (3)	10/48 (21)[a]	2/29 (7)[a]
5–6	6/47 (13)	5/29 (17)	12/47 (26)[b]	14/29 (48)[b]
7–8	4/26 (15)	14/39 (36)	10/26 (38)[b]	24/39 (62)[b]
Total[c]	16/121 (13)	20/97 (21)	32/121 (26)	40/97 (41)

[a] $\chi^2 = 1.7$.
[b] $\chi^2 = 6.4$ ($P < 0.02$).
[c] $\chi^2 = 4.7$ ($P < 0.05$).

Low but highly significant correlations were found between the magnitude of anti-$GLURP_{489–1271}$ IgG response and the magnitude of response to three RESA peptides and the native RESA as measured by erythrocyte membrane immunofluorescence (EMIF) (12) (Table 4).

Relationship of anti-$GLURP_{489–1271}$ antibodies and morbidity. To assess the effects of seropositivity in May on malaria morbidity in the subsequent transmission season, a comparison was made between asymptomatically infected children ($n = 97$) and children who suffered a clinical episode of malaria ($n = 121$), defined as fever plus parasitemia of ≥5,000 parasites per μl (Table 5). This level of parasitemia has previously been used as a criterion for clinical malaria in this epidemiological setting (9).

Overall, there was a significant association between anti-$GLURP_{489–1271}$ IgG seropositivity and reduced subsequent morbidity ($\chi^2 = 4.7$ [$P < 0.05$]), but this concealed a difference in effect according to age. For children aged 3 to 4 years, anti-$GLURP_{489–1271}$ IgG seropositivity appeared to be associated with increased morbidity, although not significantly ($\chi^2 = 1.7$).

For children aged 5 to 8 years, anti-$GLURP_{489–1271}$ IgG seropositivity was associated with reduced morbidity (MH, $\chi^2 = 6.4$ [$P < 0.02$]; odds ratio = 0.38; 95% confidence interval [0.19 and 0.76]). Anti-$GLURP_{489–1271}$ IgM seropositivity suggested a similar association with morbidity, but this was not significant overall ($\chi^2 = 1.6$) or for individual age groups. For children aged 5 to 8 years, the risk of a clinical episode of malaria decreased with increasing levels of anti-$GLURP_{489–1271}$ IgG (Fig. 3; χ^2 for trend = 4.6 [$P < 0.05$]). The numbers of children aged 3 to 4 years with a high response were too small to show any pattern. The protective effect of IgG response among 5- to 8-year-old children was confirmed by a logistic regression analysis of morbidity on $\log_e$(OD), with the confounding effects of age, sex, ethnic group, presence of any parasitemia during the May survey, sickle cell trait, and use of insecticide-impregnated bed nets during a previous intervention trial taken into account ($\chi^2 = 4.2$ [$P < 0.05$]).

DISCUSSION

The aims of this investigation were to study acquisition of anti-$GLURP_{489–1271}$ IgM and IgG antibodies as a function of age and to examine whether possession of such antibodies was correlated with protective immunity.

An age-related change in seropositivity rates and magnitudes of responses of IgM and IgG would be expected, with a high IgM seropositivity rate in younger children and a

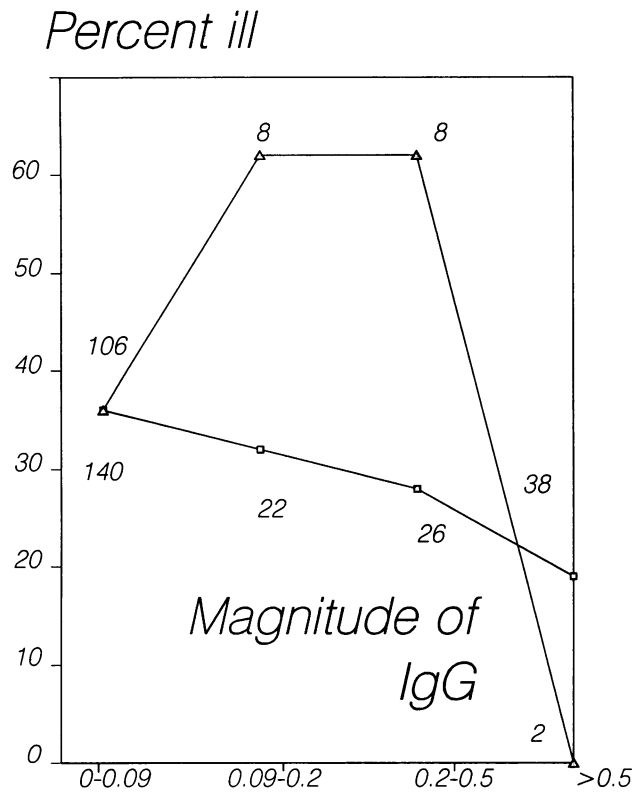

FIG. 3. Percentages of all children with clinical malaria by magnitude of the anti-GLURP$_{489-1271}$ IgG response in May (triangles, 3- to 4-year-old children; squares, 5- to 8-year-old children). Numbers of children in each group are indicated.

decrease in the IgM seropositivity rate in older children and adults due to isotype switch and the opposite pattern for IgG seropositivity. However, the present study shows that seropositivity rates and magnitudes of responses for IgM as well as for IgG increase with age. The maxima found in The Gambia are in accordance with the finding of a 55% IgM seropositivity rate (5) and a 98% IgG seropositivity rate (4) in adult Liberians. In The Gambia, exposure to *P. falciparum* of up to 15 to 20 years seems to be necessary for the majority of the population to become IgM and IgG seropositive. Seropositivity for both IgG and IgM was positively associated with parasitemia and with parasite density during the May survey. This is in contrast to the results of Högh et al. (8), who found a significantly lower parasite density in Liberian children aged 5 to 8 years with an anti-GLURP$_{489-1271}$ IgG response of high magnitude compared with parasitemic children with an antibody response of low magnitude.

The net increase in IgG-seropositive individuals as a result of seroconversion probably reflects exposure to GLURP during the rainy season. However, the finding of eight IgG-seropositive individuals converting to seronegativity suggests that the antibody response to GLURP is short-lived in the absence of reinfection by parasites. The numbers of individuals who seroconverted from IgM positivity to IgM negativity and vice versa were six and seven, respectively, reflecting the transient nature of the IgM response. The observed correlations among the magnitudes of IgG responses to GLURP$_{489-1271}$, to the three Pf155/RESA peptides, and to native Pf155/RESA in erythrocyte membrane immunofluorescence may be due to cross-reactivity of antibodies raised against similar epitopes. However, the coefficients of correlation imply that measurements for individual serum samples performed in the two assay systems had wide variations; therefore, it is unlikely that measured antibodies had identical specificities. This is in agreement with the lack of correlation found by Högh et al. (8). Instead, the correlation may be due to parallel development of antibody response against these two blood stage antigens.

The median anti-GLURP$_{489-1271}$ antibody level was higher for Fula people than for either Mandinkas or Wollofs. However, the prevalence of the antibody response was similar among all ethnic groups. High prevalences of IgG (and to some extent also IgM) antibody seropositivity tended to be associated with carriage of HLA class II alleles (*DRw8*, *DR9*, *DR7*, *DR4*, *DQw7*, and *DQw2*) which are particularly common among Fula people but rare in the other ethnic groups considered in the present study (11). Similar associations in the same population have been noted for antibody responses to epitopes of the malaria antigen Pf155/RESA (14, 15). These genetic associations may reflect preferential association of GLURP epitopes with particular class II molecules leading to optimal activation of T helper cells, or they may reflect the effects of HLA region genes which influence processes such as macrophage or B-cell activation and transport of peptides.

Description of protective immunity was based on (i) examination of blood films for the presence and density of parasites and (ii) morbidity surveillance (the presence or absence of clinical signs and symptoms consistent with malaria). These data revealed significantly more anti-GLURP$_{489-1271}$-seropositive individuals among asymptomatically infected children than among symptomatically infected children. Stratification by age revealed a difference between children aged 3 to 4 years and older children in the relation of IgG seropositivity (and magnitude of IgG response) and morbidity. Since the same magnitude of IgG response predicts different susceptibilities in different age groups, it could be that children of different ages possess qualitatively different IgG antibodies, as has been shown, for example, in the response to the bacterium *Branhamella catarrhalis* (6). Another explanation could be a change in the epitope specificities of the induced antibodies after the first years of exposure (16). Alternatively, seropositivity to GLURP$_{489-1271}$ simply might reflect exposure to *P. falciparum*, and the positive (<5 years) and negative (>5 years) associations between asymptomatic infection and seropositivity could be due to protection induced by some other factors at the age of 5 years. Therefore, the conclusion to be drawn from this study is that the development of the IgG antibody response to GLURP$_{489-1271}$ does not speak against a role in protective immunity to *P. falciparum*, whereas a positive confirmation of such a role is not provided.

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REFERENCES

1. **Borre, M. B., M. Dziegiel, E. Petersen, B. Högh, K. Rieneck, E. Riley, J. F. Meis, M. Aikawa, K. Nakamura, M. Harada, A. Wind, P. H. Jakobsen, J. Cowland, S. Jepsen, N. H. Axelsen, and J. Vuust.** 1991. Primary structure and localization of a conserved immunogenic *Plasmodium falciparum* glutamate rich protein (GLURP) expressed in both the preerythrocytic and erythrocytic stages of the vertebrate life cycle. Mol. Biochem. Parasitol. **49:**119–132.
2. **Carlsson, B., J. Wallin, J. Bohme, and E. Moller.** 1987. HLA-DR-DQ haplotypes defined by restriction fragment length analysis: correlation to serology. Hum. Immunol. **20:**93–113.
3. **Coppel, R. L., A. F. Cowman, R. F. Anders, A. E. Bianco, R. B. Saint, K. R. Lingelbach, D. J. Kemp, and G. V. Brown.** 1984. Immune sera recognize on erythrocytes a *P. falciparum* antigen composed of repeated amino acid sequences. Nature (London) **310:**789–792.
4. **Dziegiel, M., M. Borre, S. Jepsen, B. Högh, E. Petersen, and J. Vuust.** 1991. Recombinant *Plasmodium falciparum* glutamate rich protein: purification and use in enzyme linked immunosorbent assay. Am. J. Trop. Med. Hyg. **44:**306–313.
5. **Dziegiel, M., M. B. Borre, E. Petersen, B. Högh, S. Jepsen, J. Vuust, and N. Axelsen.** Capture ELISA for IgM antibodies against *Plasmodium falciparum* glutamate rich protein. J. Immunol. Methods, in press.
6. **Goldblatt, D., M. W. Turner, and R. J. Levinsky.** 1990. *Branhamella catarrhalis*: antigenic determinants and the development of the IgG subclass response in childhood. J. Infect. Dis. **162:**1128–1135.
7. **Greenwood, B. M., A. K. Bradley, A. M. Greenwood, P. Byass, K. Jammeh, S. Tulloch, F. S. J. Oldfield, and R. Hayes.** 1987. Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. Trans. R. Soc. Trop. Med. Hyg. **81:**478–486.
8. **Högh, B., E. Petersen, M. Dziegiel, K. David, A. P. Hanson, M. Borre, A. Holm, J. Vuust, and S. Jepsen.** 1992. Antibodies to a recombinant glutamate-rich *Plasmodium falciparum* protein: indirect evidence for protection in individuals living in a holoendemic area of Liberia. Am. J. Trop. Med. Hyg. **46:**307–313.
9. **Marsh, K., L. Otoo, R. J. Hayes, D. C. Carson, and B. M. Greenwood.** 1989. Antibodies to blood stage antigens of *Plasmodium falciparum* in rural Gambians and their relation to protection against infection. Trans. R. Soc. Trop. Med. Hyg. **83:**293–303.
10. **Mattei, D., K. Berzins, M. Wahlgren, R. Udomsangpetch, P. Perlmann, H. W. Griesser, A. Scherf, B. Müller-Hill, S. Bonnefoy, M. Guilotte, G. Langsley, L. Pereira da Silva, and O. Mercereau-Puijalon.** 1989. Cross-reactive antigenic determinants present on different *Plasmodium falciparum* blood-stage antigens. Parasite Immunol. **11:**15–30.
11. **Olerup, O., M. Troye-Blomberg, G. M. T. Schreuder, and E. M. Riley.** 1991. HLA-DR and -DQ gene polymorphism in West Africans is twice as extensive as in North European Caucasians: evolutionary implications. Proc. Natl. Acad. Sci. USA **88:**8480–8484.
12. **Perlmann, H., K. Berzins, M. Wahlgren, J. Carlsson, A. Björkman, M. E. Patarroyo, and P. Perlmann.** 1984. Antibodies in malarial sera to parasite antigens in the membrane of erythrocytes infected with early asexual stages of *Plasmodium falciparum*. J. Exp. Med. **159:**1686–1704.
13. **Riley, E. M., S. J. Allen, S. Bennett, P. J. Thomas, A. O'Donnell, S. W. Lindsay, M. F. Good, and B. M. Greenwood.** 1990. Recognition of dominant T cell-stimulating epitopes from the circumsporozoite protein of *Plasmodium falciparum* and relationship to malaria morbidity in Gambian children. Trans. R. Soc. Trop. Med. Hyg. **84:**648–657.
14. **Riley, E. M., S. J. Allen, M. Troye-Blomberg, S. Bennett, H. Perlmann, G. Andersson, L. Smedman, P. Perlmann, and B. M. Greenwood.** 1991. Association between immune recognition of the malaria vaccine candidate antigen Pf155/RESA and resistance to clinical disease: a prospective study in a malaria endemic region of West Africa. Trans. R. Soc. Trop. Med. Hyg. **85:**436–443.
15. **Riley, E. M., O. Olerup, S. Bennett, P. Rowe, S. J. Allen, M. J. Blackman, M. Troye-Blomberg, A. A. Holder, and B. M. Greenwood.** MHC and malaria: the relationship between HLA class II alleles. Int. Immunol., in press.
16. **Schofield, L.** 1991. On the function of repetitive domains in protein antigens of *Plasmodium* and other eukaryotic parasites. Parasitol. Today **7:**99–105.