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Synthetic Antigens Derived from *Plasmodium falciparum* Sporozoite, Liver, and Blood Stages: Naturally Acquired Immune Response and Human Leukocyte Antigen Associations in Individuals Living in a Brazilian Endemic Area

Lilian Rose Pratt-Riccio,[1]* Daiana De Souza Perce-Da-Silva,[2] Josué Da Costa Lima-Junior,[3] Evelyn Kety Pratt Riccio,[1] Marcelo Ribeiro-Alves,[4] Fátima Santos,[5] Mercia Arruda,[6] Daniel Camus,[7] Pierre Druilhe,[8] Joseli Oliveira-Ferreira,[3] Cláudio Tadeu Daniel-Ribeiro,[1] and Dalma Maria Banic[2]

[1]*Laboratório de Pesquisa em Malária, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil;* [2]*Laboratório de Imunologia Clínica, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil;* [3]*Laboratório de Imunoparasitologia, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil;* [4]*Laboratório de Pesquisa Clínica em DST/AIDS, Instituto Nacional de Infectologia Evandro Chagas, Fiocruz, Rio de Janeiro, Brazil;* [5]*Laboratório Central de Saúde Pública (LACEN), Rondônia, Brazil;* [6]*Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães, Fiocruz, Recife, Brazil;* [7]*Service de Parasitologie-Mycologie, Faculte de Médecine, Lille, France;* [8]*VAC4ALL, Paris, France*

Abstract. Peptide vaccine strategies using *Plasmodium*-derived antigens have emerged as an attractive approach against malaria. However, relatively few studies have been conducted with malaria-exposed populations from non-African countries. Herein, the seroepidemiological profile against *Plasmodium falciparum* of naturally exposed individuals from a Brazilian malaria-endemic area against synthetic peptides derived from vaccine candidates circumsporozoite protein (CSP), liver stage antigen-1 (LSA-1), erythrocyte binding antigen-175 (EBA-175), and merozoite surface protein-3 (MSP-3) was investigated. Moreover, human leukocyte antigen (HLA)-DRB1* and HLA-DQB1* were evaluated to characterize genetic modulation of humoral responsiveness to these antigens. The study was performed using blood samples from 187 individuals living in rural malaria-endemic villages situated near Porto Velho, Rondônia State. Specific IgG and IgM antibodies and IgG subclasses were detected by enzyme-linked immunosorbent assay, and HLA-DRB1* and HLA-DQB1* low-resolution typing was performed by PCR-SSP. All four synthetic peptides were broadly recognized by naturally acquired antibodies. Regarding the IgG subclass profile, only CSP induced IgG1 and IgG3 antibodies, which is an important fact given that the acquisition of protective immunity appears to be associated with the cytophilicity of IgG1 and IgG3 antibodies. HLA-DRB1*11 and HLA-DQB1*7 had the lowest odds of responding to EBA-175. Our results showed that CSP, LSA-1, EBA, and MSP-3 are immunogenic in natural conditions of exposure and that anti-EBA antibody responses appear to be modulated by HLA class II antigens.

INTRODUCTION

Malaria remains a major public health problem around the world, affecting approximately 212 million people annually in tropical areas and taking an estimated 429,000 lives, mostly those of children under 5 years of age.[1] In high-endemic malaria areas, young children are particularly susceptible, whereas with exposure, older children and adults develop considerable protection from severe malaria and death, although sterile immunity is likely never achieved.[2,3] These changes are thought to reflect the parasitological and clinical immunity, collectively referred to as naturally acquired immunity, which generally determines not only the age-specific incidence and the prevalence of infections but also the expression of pathological processes that underlie the clinical manifestations of the infection.

Because experiments demonstrating that adoptive transferred serum from naturally immune individuals protects against clinical malaria or significantly attenuates the severity and disease burden,[4,5] it is widely assumed that antibodies play an important role in malarial protective immunity. In this regard, several studies have demonstrated that cytophilic antibodies are particularly critical for immunity to malaria infection.[6–17] Therefore, efforts have been made to identify target antigens that induce antibodies in sufficient quantity and, above all, are functionally capable of participating in the development of an antimalarial immunity. Collectively, these data reflect the importance of studies of the humoral immune response to malaria vaccine candidates in individuals naturally exposed to malaria infection. Moreover, this type of evaluation is a crucial point for field trials of potentially protective vaccine candidates.

In this scenario, studies assessing the immune response against *Plasmodium falciparum* vaccine candidates in naturally exposed individuals from the Brazilian Amazon were conducted by our group.[10,18–22] In Brazil, malaria is hypo- to meso-endemic, present throughout the year with clear seasonal fluctuations and is frequently associated with migration movements of nonimmune individuals to areas where malaria is endemic.[23] The exposed populations are composed of autochthonous and migrant individuals from the nonendemic regions of Brazil. Asymptomatic and or paucisymptomatic infections by *P. falciparum* and *Plasmodium vivax* were detected in epidemiological studies in the states of Rondônia (RO) and Amazonas, which likely indicates a pattern of clinical immunity in both populations.[24–28] Seropidemiological studies investigating the type of immune responses elicited in naturally exposed populations to several malaria vaccine candidates in Brazilian populations provide important information on whether immune responses specific to these antigens are generated in natural infections and their immunogenic potential as vaccine candidates.

In the present work, the natural acquired humoral immune response against four synthetic antigens derived from *P. falciparum* sporozoites, liver and blood stage antigens in malaria-exposed individuals from the Brazilian Amazon was analyzed, and HLA-DRB1* and -DQB1* were used as

* Address correspondence to Lilian Rose Pratt-Riccio, Laboratório de Pesquisa em Malária, Instituto Oswaldo Cruz, Fiocruz, Pavilhão Leônidas Deane, sala 515, Avenida Brasil 4365, Manguinhos, Rio de Janeiro, Brazil 21040-360. E-mail: riccio@ioc.fiocruz.br

molecular markers in an attempt to determine the presence of a genetic modulation of the humoral responsiveness to the *P. falciparum* antigens. The identification of antigens recognized by antibodies of such individuals may help to establish the best targets for a potential multipeptide-based vaccine for future use in this population.

MATERIALS AND METHODS

Study area and subjects. The study described here was performed using blood samples obtained from individuals from rural malaria-endemic villages situated near Porto Velho, the capital of the state of RO, and a malaria-endemic region in the Brazilian Amazon (63°54′13″W 8°45′43″S). The population of Porto Velho, sampled in this study, is composed both of autochthonous individuals and migrants from several nonendemic areas of Brazil who have lived in the region for 10 years or more.

Serum samples were obtained from 187 malaria exposed (autochthonous and migrant) individuals (77 women and 110 men) with ages ranging from 8 to 74 years (average age 31 ± 16 years). The average time of residence in the malaria-endemic area was 24 ± 16 years. These individuals composed the exposed group. Additionally, serum samples from 109 individuals (61 women and 48 men with an average age of 38 ± 10 years) living in downtown Porto Velho, where malaria transmission does not occur, were included in our study as noninfected individuals. These individuals composed the nonexposed group. This population was also composed of autochthonous and Brazilian migrants inhabiting the region for 26 ± 18 years. All of the individuals from the nonexposed group were negative for malaria parasites as assessed by thick blood films. Most of the individuals (64%) denied prior malaria infection, and 36% reported an average of 1.8 ± 1.4 past episodes of malaria occurring at least 5 years before the collection of the samples. Nonendemic control blood samples from 15 individuals of the laboratory staff (Rio de Janeiro, Brazil) who had neither a history of malaria nor contact with malaria transmission were included in our study as controls. Written informed consent was obtained from all of the individuals before admission to the study. This study was reviewed and approved by the Oswaldo Cruz Foundation Ethical Committee (258/04).

The samples and the survey data were collected 2–4 times during the dry months (June to August), from 1996 to 2007, coinciding with the period of increased malaria transmission in the RO State. The longitudinal study of antibody response allowed us to discriminate more precisely between responders and nonresponders. To evaluate the degree of exposure to malaria, the survey data used were age, time of residence in the endemic area and the number of malaria episodes reported by each individual.

Venous peripheral blood (10 mL) was collected into EDTA tubes for both an antibody analysis and HLA class II typing. The plasma was stored at −20°C, and the pellets, containing peripheral blood cells, were mixed with equal volumes of a cryopreservation solution (0.9% NaCl/4.2% sorbitol/20% glycerol) and were stored in liquid nitrogen until use. Thin and thick blood smears were examined for the identification of the malaria parasite by a technician experienced in malaria diagnosis from the Brazilian Malaria Health Services and from the Laboratory of Malaria Research (Fiocruz), which is the headquarters of the Center for Malaria Research and Training, a reference center for malaria diagnosis in the Extra-Amazonian Region for the Brazilian Ministry of Health. Thick blood smears from all of the subjects were stained with Giemsa, and a total of 200 microscopic fields were examined under a 1,000-fold magnification. Thin blood smears of the positive samples were examined for species identification. The parasite density was determined by counting the parasites in a predetermined number of white blood cells in the thick blood films, and the number of blood parasites per milliliter was calculated.[29] To increase the sensitivity of the parasite detection, molecular analyses using specific primers for genus (*Plasmodium* sp.) and species (*P. falciparum* and *P. vivax*) were performed in all of the samples as previously described.[30] Donors positive for *P. vivax* and/or *P. falciparum* at the time of blood collection were subsequently treated per the chemotherapeutic regimen recommended by the Brazilian Ministry of Health.

Synthetic peptides. The evaluation of the antibody response was performed using synthetic peptides derived from *P. falciparum* sporozoite-, liver- and blood-stages antigens. Peptides were synthesized by fluorenylmethoxycarbonyl (F-moc) solid-phase chemistry.[31] Analytical chromatography of the peptides demonstrated a purity of > 90%. Peptide sequences were the following:

Circumsporozoite protein. A long synthetic peptide consisting of 50 repeat sequences of asparagine–alanine–asparagine–proline of the circumsporozoite protein (CSP), which is the predominant surface antigen on the sporozoites (GenBank: AKU89585.1)

Liver stage antigen-1. A 41-mer peptide containing the epetitive epitope of the pre-erythrocytic stage Liver Stage Antigen-1 (LSA-1). The sequence is LAKEKLQEQQS DLEQERLAKEKLQEQQSDLEQERLAKEKLQ (GenBank: CZT98619.1

Erythrocyte binding antigen. A 42-mer peptide containing region IV of the erythrocyte binding antigen 175 ($EBA\text{-}175_{1089\text{-}1130}$). The sequence is SNNEYKVNERE DERTLTKEYEDIVLKSHMNRESDDGELYDEN (GenBank: 001349207)

Merozoite surface protein-3. A 27-mer peptide containing the central region of the merozoite surface protein-3 ($MSP\text{-}3_{211\text{-}237}$). The sequence is AKEASSYDYILGWEFGGG VPEHKKEEN (GenBank: CZT98611.1)

Enzyme-linked immunosorbent assay. Microtiter 96-well plates (Maxisorp, NUNC, Denmark) were coated with the antigens at an optimal dilution using phosphate-buffered saline at pH 7.4 (PBS) or a carbonate-bicarbonate buffer at pH 9.6 at 100 μL/well overnight at 4°C (see Table 1). The plates were washed, and the uncoated sites were blocked and then reacted for 1 hour with sera in duplicate that was diluted (1/100) in dilution buffer. The plates were washed, and mouse antihuman IgG or IgM (Sigma, St. Louis, MO) was diluted 1/2,000 in dilution buffer and incubated for 1 hour. To detect specific IgG subclasses plates were incubated with mouse anti-human IgG1, IgG2, IgG3, or IgG4 antibodies peroxidase conjugate (*Sigma*) diluted 1/1,000 in dilution buffer for 2 hours. After washing, 100 μL of a solution of orthophenylenediamine (Sigma) and H_2O_2 (Sigma) in citrate-phosphate buffer at pH 5.0 was added to each well, and the plate was incubated for 15–30 minutes at room temperature in the dark, and then 50 μL/well of H_2SO_4 (Sigma)

TABLE 1
Antigens and enzyme-linked immunosorbent assay antibody assays

Antigen	Coating	Washing	Blocking	Serum	Anti-serum
(NANP)50	1 μg/mL in PBS	3 times with PBST	1 hour with 5% (wt/vol) BSA in PBST at 37°C	1 hour diluted in 2.5% (wt/vol) BSA in PBST at 37°C	Diluted in 2.5% (wt/vol) BSA in PBST at 37°C
LSA-1	2 μg/mL in PBS	3 times with PBST	Overnight with 2.5% (wt/vol) powdered-milk in PBS at RT	1 hour diluted in 1.25% (wt/vol) powdered milk in PBS at RT	Diluted in 1.25% (wt/vol) powdered milk in PBS at RT
EBA	2 μg/mL in carbonate-bicarbonate buffer	3 times with PBST	1 hour with 5% (wt/vol) powdered-milk in PBST at 37°C	1 hour diluted in 1.25% (wt/vol) powdered milk in PBST at 37°C	Diluted in 1.25% (wt/vol) powdered milk in PBST at 37°C
MSP-3	2 μg/mL in carbonate-bicarbonate buffer	3 times with PBST	1 hour with 3% (wt/vol) powdered-milk in PBST at RT	1 hour diluted in 1% (wt/vol) powdered milk in PBST at RT	Diluted in 1% (wt/vol) powdered milk in PBST at RT

EBA-157 = erythrocyte binding antigen-175; LSA-1 = liver stage antigen-1(LSA-1); MSP-3 = merozoite surface protein-3; PBST = PBS-Tween 20 0.05%; wt/vol = weight/volume; RT = room temperature.

2 N was used to stop the reaction. The plates were read at 492 nm in a spectrophotometer (Spectramax 250, Molecular Devices). Sera from the 15 Rio de Janeiro controls were used to establish the normal range for the assay. The cut-off value was determined as the mean optical density (OD) + 3 standard deviations of the Rio controls. To standardize the OD data obtained in the different experiments, an OD index was calculated for each immunoglobulin determination as the ratio of the observed OD/cut-off values. Samples with an OD index > 1.0 were considered positive. The cut off values for CSP, LSA-1, EBA, and MSP-3 were, respectively, 0.094, 0.089, 0.067, and 0.075 for IgG, 0.195, 0.164, 0.101, and 0.131, for IgM, 0.083, 0.086, 0.084, and 0.072 for IgG1, 0.179, 0.151, 0.104, and 0.131 for IgG2, 0.099, 0.105, 0.120, and 0.128, for IgG3 and 0.109, 0.110, 0.123, and 0.18 for IgG4.

HLA typing. HLA typing was performed in the 107 malaria naturally exposed individuals and the 77 malaria nonexposed individuals. Genomic DNA was isolated from frozen peripheral blood by the phenol-chloroform extraction procedure as previously described.[32] HLA-DRB1* and –DQB1* low-resolution typing was performed by polymerase chain reaction with sequence-specific primers on all of the samples as previously described.[33,34]

Statistical analysis. The data were stored in the dBASE data bank software (Ashton Tate, Borland, CA). Statistica (Microsoft, Redmond, WA) and the EpiInfo version 6 (Centers for Disease Control and Prevention, Atlanta, GA) statistical software programs were used for the data analysis. Student's *t* test was used to analyze the differences in the mean values, and χ^2 analysis was applied to compare the prevalence of the positive responses. The Spearman rank coefficient test was used to analyze the correlation between the variables. The antigen frequencies were calculated by the formula $af = n/N$, where n is the number of samples positive for the antigen and N is the total number of samples, and the gene frequencies were calculated by the formula $gf = 1 - \sqrt{(1 - af)}$.[35] The HLA antigen-specific associations with the responders and nonresponders were analyzed by multiple logistic regression using R version 2.14.0 statistical software (The R Foundation for Statistical Computing, Vienna, Austria, available at http://www.R-project.org/) and were corrected by the time of residence in a malaria-endemic area and the number of previous malaria attacks. The HLA unidentified specificities (blank) as well as the HLA specificities with frequencies equal to zero in at least one of the studied groups were pooled into one group as other specificities.

RESULTS

Clinical and epidemiological characteristics of the studied population. Individuals from the exposed group claimed to have experienced an average of 1.2 malaria episodes in the previous 12 months. All except 13 individuals reported at least one malaria episode during their life, and 44 (23.5%) of the subjects reported more than 10 episodes. Forty-eight (26%) of the individuals had detectable parasitemia (4,389 ± 9,610 parasites/μL) at the time of the blood sampling, and 37 were infected with *P. falciparum,* nine were infected with *P. vivax* and two were infected with *P. falciparum* and *P. vivax*.

IgG and IgM antibody responses against CSP, LSA-1, EBA, and MSP-3. The prevalence of individuals presenting antibodies, regardless of their IgM or IgG type and IgG isotype against CSP, LSA-1, EBA, and MSP-3 were, respectively, 67% (125/187), 73% (136/187), 78% (146/187), and 61% (115/187). Antibodies against EBA and LSA-1 were significantly more prevalent than antibodies against MSP-3 (χ^2 = 11.413; $P = 0.0007$, MSP-3 × EBA; $\chi^2 = 4.693$; $P = 0.03$, MSP-3 × LSA-1). The antibody responses to CSP, LSA-1, EBA, and MSP-3 were strongly associated with the time of residence in a malaria-endemic area (CSP: $P = 0.01$; LSA-1: $P = 0.0003$; EBA: $P = 0.01$; MSP-3: $P = 0.01$) (Table 2).

The frequencies of individuals presenting IgG and IgM antibodies are shown in Figure 1. IgG antibodies against EBA were significantly more prevalent (70%) than antibodies against LSA1 (45%), MSP-3 (41%), and CSP (38%) ($P < 0.0001$, χ^2 – 22.191, EBA × LSA-1; $P < 0.0001$, $\chi^2 = 31.548$, EBA × MSP-3; $P < 0.0001$, $\chi^2 = 37.573$, EBA × CSP). Not only was the frequency of the responders to EBA higher, but EBA also induced higher levels of IgG antibodies than LSA-1, MSP-3, and CSP ($P = 0.03$, EBA × LSA-1; $P = 0.0006$, EBA × MSP-3; $P < 0.0001$, EBA × CSP) (Figure 2). The frequency of the responders showed that IgM antibodies to LSA-1 were significantly more prevalent (68%) than IgM antibodies to EBA (48%), MSP-3 (55%), and CSP (57%) ($P < 0.0001$, $\chi^2 = 15.852$, LSA-1 × EBA; $P = 0.01$, $\chi^2 = 6.521$, LSA-1 × MSP-3; $P = 0.02$, $\chi^2 = 4.944$, LSA-1 × CSP). Interestingly, MSP-3 showed higher levels of an IgM antibody response than EBA, LSA-1, and CSP ($P < 0.0001$, MSP-3 × EBA; $P = 0.03$, MSP-3 × LSA-1; $P < 0.0001$, MSP-3 × CSP) (Figure 2). The levels of IgG and IgM antibodies to LSA-1, EBA, and MSP-3 increased with age (LSA-1: $P = 0.02$, $r = 0.2433$ for IgG, $P = 0.006$, $r = 0.2384$ for IgM; EBA: $P = 0.007$, $r = 0.2344$ for IgG, $P = 0.001$, $r = 0.3343$ for

TABLE 2
Characteristics of the CSP, LSA-1, EBA, and MSP-3 responders and non-responders in the exposed group

	CSP		LSA-1		EBA		MSP-3	
	R	NR	R	NR	R	NR	R	NR
N	125/187 (66.8%)	62/187 (33.2%)	136/187* **(72.7%)**	51/187 (27.3%)	146/187** **(78.1%)**	41/187 (21.9%)	115/187 (61.5%)	72/187 (38.5%)
Sex								
Male (%)	63.2	50	61.5	52.9	61.6	48.8	58.3	59.7
Female (%)	36.8	50	38.5	47.1	38.4	51.2	41.7	40.3
Age[Ω]	32 ± 16	30 ± 16	32 ± 16	29 ± 14	32 ± 16	31 ± 14	34 ± 16	28 ± 14
Time of residence in malaria-endemic area†	26 ± 16***	21 ± 16	27 ± 19****	18 ± 13	26 ± 17*****	20 ± 13	28 ± 17‡	19 ± 13
Reported number of previous malaria attacks	15 ± 16	13 ± 15	15 ± 16	14 ± 14	14 ± 15	15 ± 17	16 ± 16	13 ± 14

CSP = circumsporozoite protein; EBA-157 = erythrocyte binding antigen-175; HLA = human leukocyte antigen; LSA-1 = liver stage antigen-1(LSA-1); MSP-3 = merozoite surface protein-3; N = number of positive individuals/number of tested individuals; R = responders; NR = nonresponders; † = years. * $P = 0.0007$, LSA-1 vs MSP-3; ** $P = 0.03$, EBA vs MSP-3; *** $P = 0.01$, CSP responders vs nonresponders; **** $P = 0.0008$, LSA-1 responders vs nonresponders; ***** $P = 0.04$, EBA responders vs nonresponders; ‡ MSP-3 responders vs nonresponders.

IgM; MSP-3: $P = 0.03$, $r = 0.2451$ for IgG, $P = 0.004$, $r = 0.2767$ for IgM) and time of residence in a malaria-endemic area (LSA-1: $P = 0.02$, $r = 0.2510$ for IgG, $P = 0.006$, $r = 0.2407$ for IgM; EBA: $P = 0.03$, $r = 0.1822$ for IgG, $P = 0.005$, $r = 0.2944$ for IgM; MSP-3: $P = 0.001$, $r = 0.3693$ for IgG, $P = 0.01$, $r = 0.3464$ for IgM).

Twenty-one of the 109 individuals (19.3%) from the non-exposed group presented antibodies against *P. falciparum* antigens. Of these, 16 individuals (14.7%) presented antibodies against a single antigen, three individuals (2.7%) presented antibodies against two antigens and two individuals (1.8%) presented antibodies against three antigens. The frequencies of the antibodies against individual antigens were low: 10 (9.2%) individuals presented antibodies against CSP, 10 (9.2%) presented antibodies against LSA-1, five (4.6%) presented antibodies against EBA and four (3.7%) presented antibodies against MSP-3. None of the 15 Rio-controls, who had neither a history of malaria nor contact with malaria transmission areas, had detectable antibodies to CSP, LSA-1, EBA, or MSP-3 (OD index values for IgG and IgM, respectively: CSP: 0.586 and 0.765; LSA-1: 0.476 and 0.698; EBA: 0.627 and 0.821; MSP-3: 0.611 and 0.876).

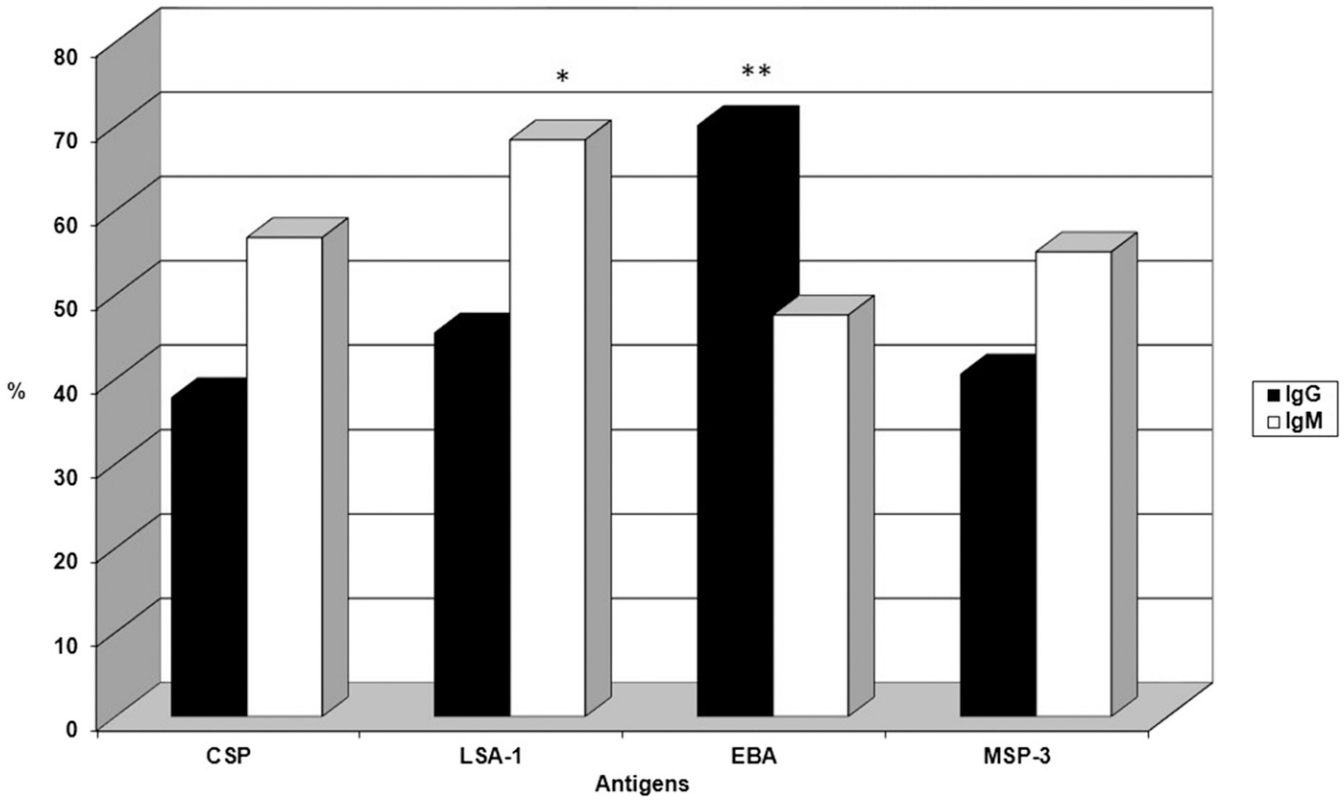

FIGURE 1. Frequency of individuals from the exposed group with IgG and IgM antibodies to circumsporozoite protein (CSP), liver stage antigen-1 (LSA-1), erythrocyte binding antigen (EBA), and merozoite surface protein-3 (MSP-3) antigens. * $P < 0.0001$, IgM LSA-1 vs IgM EBA; $P = 0.02$, IgM LSA-1 vs IgM CSP; $P = 0.01$, IgM LSA-1 vs IgM MSP-3; ** $P < 0.0001$, IgG EBA vs IgG CSP, IgG LSA-1, and IgG MSP-3.

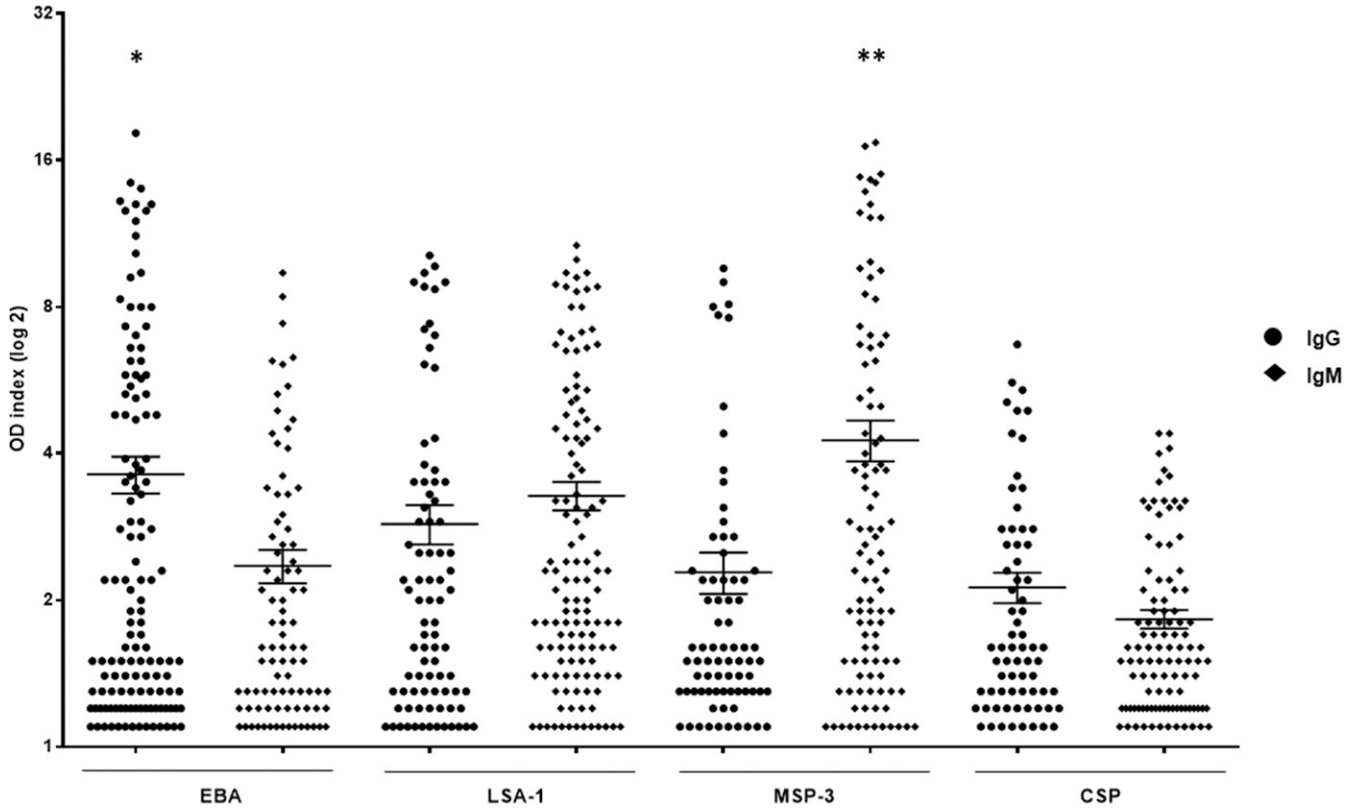

FIGURE 2. Distribution of the IgG and IgM antibody response (OD index) against erythrocyte binding antigen (EBA), liver stage antigen-1 (LSA-1), merozoite surface protein-3 (MSP-3), and circumsporozoite protein (CSP) in the exposed group. * $P < 0.0001$, IgG EBA vs IgG CSP; $P = 0.03$, IgG EBA vs IgG LSA-1; $P = 0.0006$, IgG EBA vs IgG MSP-3. ** $P < 0.0001$, IgM MSP-3 vs IgM EBA; $P < 0.0001$, IgM MSP-3 vs IgM CSP; $P = 0.03$, IgM MSP-3 vs IgM LSA-1.

IgG isotypes against CSP, LSA-1, EBA, and MSP-3. IgG isotypes were analyzed in all of the IgG positive samples. The frequencies of the individuals presenting IgG1, IgG2, IgG3, and IgG4 antibodies against CSP, LSA-1, EBA, and MSP-3 are shown in Figure 3. The results show that CSP mainly induced cytophilic IgG1 and IgG3 antibodies, and LSA-1 and MSP-3 preferentially induced an IgG1 and IgG4 antibody response, whereas EBA mainly induced an IgG1 antibody response ($P < 0.05$, for all analyses). As shown in Figure 4, EBA and LSA-1 induced higher levels of IgG1 antibodies, whereas CSP induced higher levels of IgG3 antibodies, and MSP-3 induced higher levels of IgG4 antibodies. The levels of IgG1 antibodies against LSA-1 and EBA and the levels of IgG3 antibodies against CSP showed a positive correlation with age (LSA-1: $P = 0.04$, r = 0.2344; EBA: 0.004, r = 0.2764; CS$P = 0.03$, r = 0.2656). No correlation between the level of the isotypes and the time of exposure or parasitemia was detected.

HLA class II typing. The majority of the individuals from the exposed group (107/187–57%) and from the nonexposed group (77/109–70%) were typed for HLA class II, and there were no differences in the HLA-DR and HLA-DQ antigen frequencies between these groups ($\chi^2 = 23.376$, df = 14, $P > 0.05$ for HLA-DR; ($\chi^2 = 6.064$, df = 7, $P > 0.05$ for HLA-DQ; data not shown). To evaluate the effect of class II antigens on the immune responses to CSP, LSA-1, EBA, and MSP-3 antigens in the exposed group, the individuals were regrouped into responder and non-responder groups. The individuals from the exposed group who were classified as nonresponders were those who had no detectable antibodies in the different timepoints in the study period. Using a multiple logistic regression analysis, corrected for the time of residence in a malaria-endemic area and the number of previous malaria attacks, it was possible to observe that HLA-DRB1*11 (OR = 0.16; *P* value: 0.006) and HLA-DQB1*7 (OR: 0.24; *P* value: 0.034) had the lowest odds ratios for responding to EBA-175 (Table 3).

DISCUSSION

Over the years, peptide vaccine strategies using *P. falciparum* derived antigens have reemerged as an attractive approach against malaria in Africa. However, few studies have been conducted on malaria-exposed populations from non-African countries, particularly in Brazil. Therefore, the seroepidemiological profiles of the naturally exposed individuals from a malaria-endemic setting of the Brazilian Amazon against four synthetic peptides derived from the main vaccine candidates against *P. falciparum* were investigated. The association between the immunological recognition of a given antigen and a certain degree of protection against malaria may indicate the potential value of the antigen as a candidate vaccine. Moreover, is important to identify the baseline of the naturally acquired immune response to distinguish it from the vaccine-induced response in future clinical trials.[36]

The results indicate that all four synthetic peptides were broadly recognized by naturally acquired antibodies and that these antibody responses were associated with the time

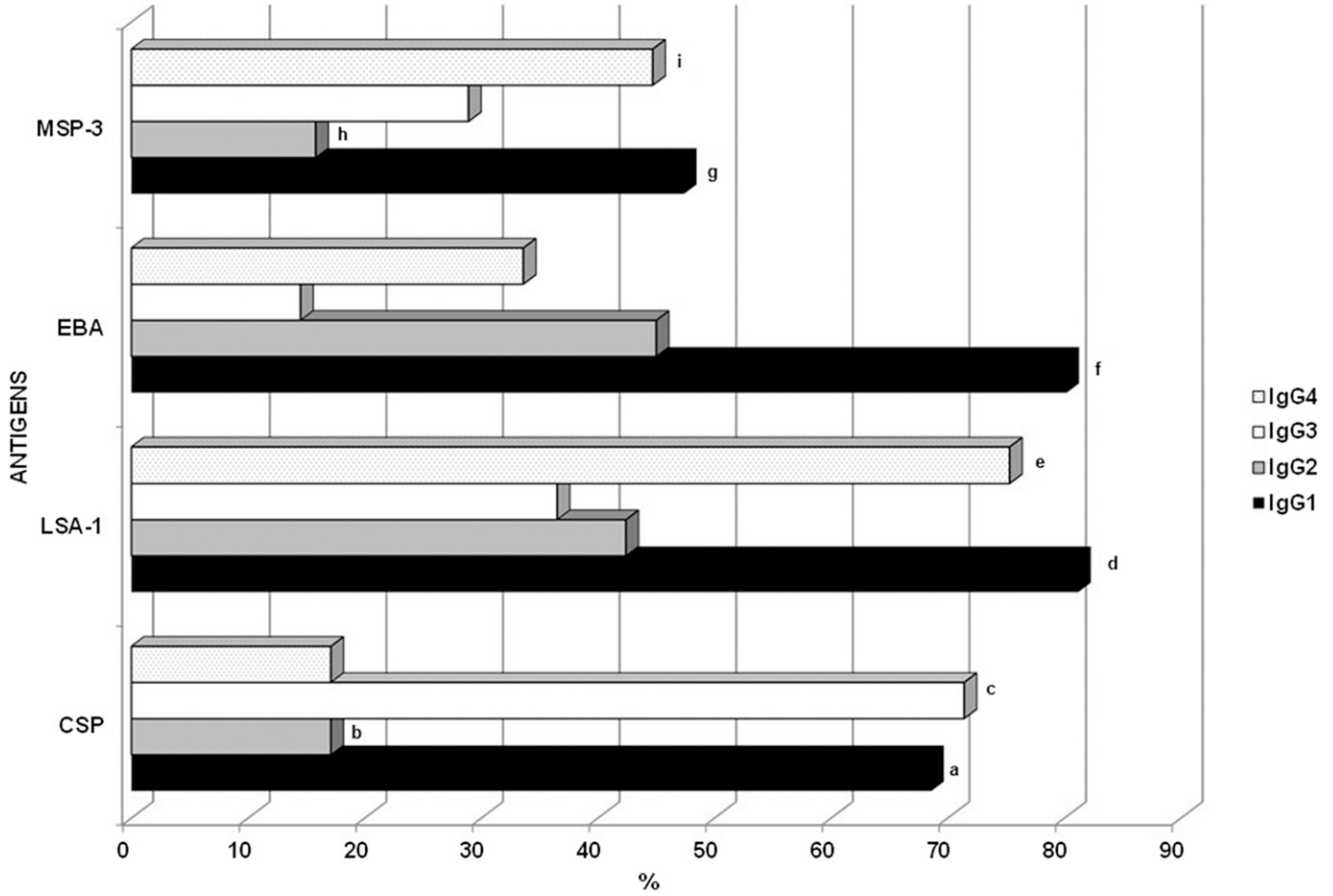

FIGURE 3. Frequency of the individuals from the exposed group with IgG1, IgG2, IgG3 and IgG4 antibodies against the circumsporozoite protein (CSP), liver stage antigen-1 (LSA-1), erythrocyte binding antigen (EBA), and merozoite surface protein-3 (MSP-3) antigens. a: $P < 0.0001$, CSP IgG1 × CSP IgG2 and CSP IgG4; b: $P < 0.0001$, CSP IgG2 × EBA IgG2; $P = 0.0009$, CSP IgG2 × LSA-1 IgG2; c: $P < 0.0001$, CSP IgG3 × CSP IgG2 and CSP IgG3 × CSP IgG4; $P < 0.0001$, CSP IgG3 × LSA-1 IgG3, MSP-3 IgG3, and EBA IgG3; d: $P < 0.0001$, LSA-1 IgG1 × LSA-1 IgG2 and LSA-1 IgG3; $P = 0.0001$, LSA-1 IgG1 × MSP-3 IgG1; e: $P < 0.0001$, LSA-1 IgG4 × LSA-1 IgG2 and LSA-1 IgG3; $P < 0.0001$, LSA-1 IgG4 × CSP IgG4, EBA IgG4, and MSP-3 IgG4; f: $P < 0.0001$, EBA IgG1 × EBA IgG2, EBA IgG3 and EBA IgG4; $P = 0.0001$, EBA IgG1 × MSP-3 IgG1; g: $P < 0.0001$, MSP-3 IgG1 × MSP-3 IgG2; $P = 0.02$, MSP-2 IgG1 × IgG3; h: $P < 0.0001$, MSP-3 IgG2 × EBA IgG2; $P = 0.0003$, MSP-3 IgG3 × LSA-1 IgG2; i: $P = 0.0002$, MSP-3 IgG4 × MSP-3 IgG2.

of residence in a malaria-endemic area, indicating that CSP, LSA-1, EBA, and MSP-3 are immunogenic in natural conditions of exposure and appear to be dependent of the time of exposure. Curiously, no association was recorded between the anti-CSP, -LSA-1, -EBA, and -MSP-3 antibody prevalence and the number of previous malaria episodes in the studied population. This finding may be limited by the donor-reported data, particularly for individuals born in the area who do not recall childhood infections. Moreover, people living for longer periods of time in the region may have acquired some degree of immunity to the clinical disease after experiencing a number of infections and, therefore, report less episodes of clinical malaria in the more recent years, leading to a bias in the analysis. In fact, studies have reported a high frequency of asymptomatic malaria infections among individuals in this area.[25,26,37–40] In this regard, the population in the present study has been living in the Amazon region for an average of 24 years. Most likely, given the high prevalence of *P. vivax* in relation to *P. falciparum* in the area, the number of malaria infections does not necessarily mean stimulation of responses to *P. falciparum* antigens.

IgG antibodies to a number of pre-erythrocytic and erythrocytic malaria vaccine candidates are associated with protection against malaria in areas with different levels of transmission.[10,13,19,41–46] In the present study, the results showed that both the prevalence of the responders and the levels of IgG antibodies were higher for EBA than for LSA1, MSP-3, and CSP. These results differ from those in a study performed by Ford et co-workers who observed a low immunoreactivity to EBA-175 in individuals living in different states of the Brazilian Amazon region, including Mato Grosso, Amapá, Rondonia, Pará, and Acre.[47] These differences may be a result of dissimilarities in exposure, age, and time of residence of the studied populations in the endemic area because several studies have demonstrated a direct effect of these parameters on the antibody response to several plasmodial antigens.[18,19,45,48] Given that the repertoire of the host erythrocyte receptors available in specific populations will select for the expression of specific parasite ligands that can mediate a successful erythrocyte invasion, the immune response to such ligands may vary in different populations, correlating to their expression. We can also hypothesize that

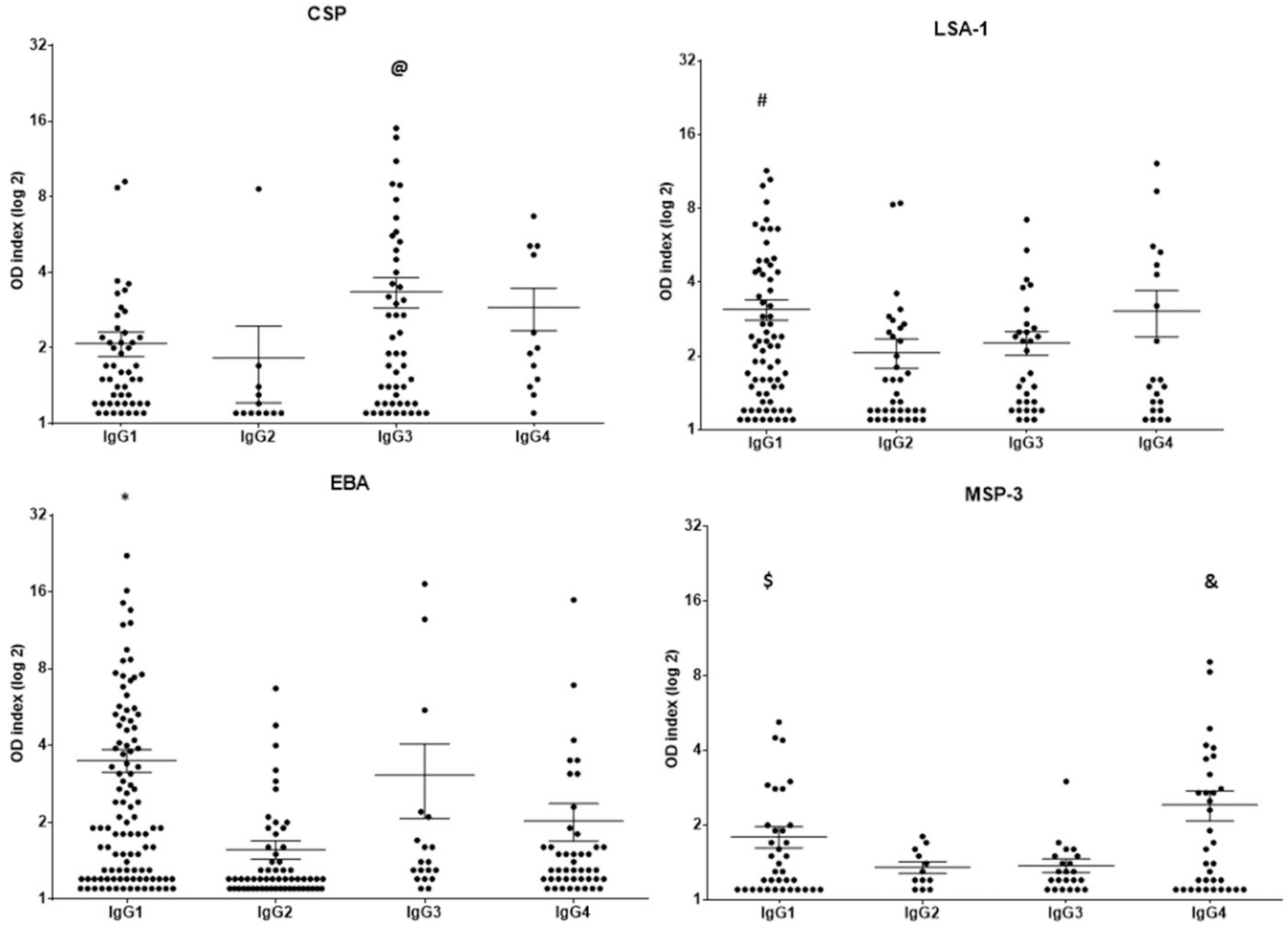

FIGURE 4. Distribution of the IgG1, IgG2, IgG3, and IgG4 antibody response (OD index) against the circumsporozoite protein (CSP), liver stage antigen-1 (LSA-1), erythrocyte binding antigen (EBA), and merozoite surface protein-3 (MSP-3) antigens in the exposed group. @ $P = 0.01$, CSP IgG3 vs CSP IgG1. # $P = 0.01$, LSA-1 IgG1 vs LSA-1 IgG2; $P = 0.03$, LSA-1 IgG1 vs LSA-1 IgG3. * $P < 0.0001$, EBA IgG1 vs EBA IgG2; $P = 0.003$, EBA IgG1 vs EBA IgG4). $ $P = 0.02$, MSP-3 IgG1 vs MSP-3 IgG2; $P = 0.03$, MSP-3 IgG1 vs MSP-3 IgG3. & $P = 0.003$, MSP-3 IgG4 vs MSP-3 IgG2; $P = 0.004$, MSP-3 IgG4 vs MSP-3 IgG3.

the difference in immunoreactivity may be due the differential usage of the EBA-175-glycophorin A invasion pathway. Interestingly, the prevalence of IgG antibodies against EBA-175 in our study was similar to that observed in Gambia and Nigeria.[49] Notably, high levels of IgG antibodies against EBA have been consistently associated with protection from clinical malaria in previous studies.[15,50–52]

IgM antibodies to LSA-1 were significantly more prevalent than IgM antibodies to EBA, MSP-3 and CSP. A high frequency of IgM antibodies against LSA-1 was observed in individuals living in Kenya; however, in this study, IgM antibodies to LSA-1 were more frequent in individuals with parasitemia than in individuals without parasitemia.[53] In our study, no association was observed between the IgM antibodies against LSA-1 and the presence or levels of parasitemia. The elevated prevalence of IgM against LSA-1 in this population may be related with the genetic background of the studied individuals. A recent study compared the breadth and magnitude of *P. falciparum*-specific IgM and IgG responses in two distinct ethnic groups, Fulani and Dogon, with a microarray containing 1,087 *P. falciparum* proteins. The authors observed that IgM response more strongly distinguished the two ethnic groups. Therefore, the authors suggest that the selective pressure of malaria may result in host genetic polymorphisms that yield more robust IgM responses in certain ethnic groups, influencing the antigen specificity of IgM responses to infection.[54]

The specific antibodies induced by natural infections to most proteins are associated with age and time of exposure in endemic regions, a phenomenon that has been frequently reported for various antigens.[10,18,19,41–46,55,56] In the present work, the levels of IgG and IgM antibodies to LSA-1, EBA, and MSP-3 increased with age and time of residence in a malaria-endemic area, reflecting the most likely exposure to the malaria parasites and, possibly, the maturation of the immune system over time.

Evidence that protective immunity to *P. falciparum* malaria is associated with different classes and subclasses of antibodies reveals the importance of considering the quality of the response. Cytophilic IgGs play an important role in protection against malarial disease.[6,57–61] The acquisition of protective immunity is associated with the cytophilicity of IgG1 and IgG3 antibodies and the reduced proportion of IgG2 and IgG4 noncytophilic subclasses of the same specificity that can

TABLE 3

Distribution of the HLA II alleles (DRB1 and DQB1) and the odds ratios (ORs) for the association among the HLA alleles and antigens

HLA alleles			EBA-175				MSP3				LSA-1				CSP			
			NR (%) *N* = 17	R (%) *N* = 91	OR (95% CI)	*P* value	NR (%) *N* = 36	R (%) *N* = 72	OR (95% CI)	*P* value	NR (%) *N* = 23	R (%) *N* = 85	OR (95% CI)	*P* value	NR (%) *N* = 22	R (%) *N* = 86	OR (95% CI)	*P* value
DR	*01	Y	15 13.9)	78 (72.2)	1.13 (0.21–6.13)	0.881	32 (29.6)	61 (56.4)	1.97 (0.49–7.96)	0.337	18 (16.6)	75 (69.4)	0.53 (0.15–1.88)	0.334	17 (15.7)	77 (71.3)	0.4 (0.11–1.44)	0.166
		N	2 (1.8)	13 (12)			4 (3.7)	11 (10.1)			5 (4.6)	10 (9.2)			5 (4.6)	9 (8.3)		
	*04	Y	15 (13.9)	65 (60.2)	2.9 (0.59–14.53)	0.186	28 (25.9)	52 (48.1)	1.47 (0.53–4.1)	0.452	18 (16.6)	62 (57.4)	1.58 (0.51–4.89)	0.427	18 (16.6)	63 (58.3)	1.8 (0.53–6.05)	0.342
		N	2 (1.8)	26 (24.1)			8 (7.4)	20 (18.5)			5 (4.6)	23 (21.3)			4 (3.7)	23 (21.3)		
	*07	Y	14 (13)	78 (72.2)	0.65 (0.14–2.97)	0.588	34 (31.4)	58 (53.7)	3.76 (0.72–19.47)	0.113	21 (19.4)	72 (66.6)	1.88 (0.36–9.61)	0.446	18 (16.6)	73 (67.5)	0.61 (0.16–2.27)	0.463
		N	3 (2.8)	13 (12.04)			2 (1.8)	14 (12.9)			2 (1.9)	13 (12)			4 (3.7)	13 (12)		
	*08	Y	16 (14.8)	76 (70.4)	1.79 (0.20–15.94)	0.598	30 (27.7)	62 (57.4)	0.3 (0.08–1.15)	0.082	22 (20.3)	70 (64.8)	3.43 (0.41–28.66)	0.254	21 (19.4)	71 (65.7)	2.89 (0.34–24.35)	0.328
		N	1 (0.9)	15 (13.8)			6 (5.5)	10 (9.2)			1 (0.9)	15 (13.8)			1 (0.9)	15 (13.8)		
	*09	Y	16 (14.8)	86 (79.6)	0.61 (0.05–6.63)	0.687	34 (31.4)	68 (62.9)	0.51 (0.06–3.92)	0.522	22 (20.3)	81 (75)	0.73 (0.06–8.03)	0.803	21 (19.4)	80 (74.)	1.3 (0.14–12)	0.812
		N	1 (0.9)	5 (4.6)			2 (1.8)	4 (3.7)			1 (0.9)	4 (3.7)			1 (0.9)	6 (5.5)		
	*10	Y	_				35 (32.4)	71 (65.7)	1.85 (0.1–33.44)	0.676	22 (20.3)	84 (77.7)	0.38 (0.02–6.81)	0.517	21 (19.4)	85 (78.7)	0.42 (0.02–7.74)	0.563
		N					1 (0.9)	1 (0.9)			1 (0.9)	1 (0.9)			1 (0.9)	1 (0.9)		
	*11	Y	**9 (8.3)**	**77 (71.3)**	**0.16 (0.04–0.59)**	**0.006**	27 (25)	59 (54.6)	0.63 (0.22–1.8)	0.397	19 (17.5)	73 (67.5)	0.89 (0.25–3.17)	0.867	17 (15.7)	70 (64.8)	0.75 (0.23–2.4)	0.633
		N	**8 (7.4)**	**14 (12.9)**			9 (8.3)	13 (12)			4 (3.7)	12 (11.1)			5 (4.6)	16 (14.8)		
	*12	Y	15 (13.8)	84 (77.7)	0.91 (0.09–8.83)	0.937	32 (29.6)	67 (62)	0.96 (0.18–5.13)	0.966	22 (20.3)	78 (72.2)	1.71 (0.18–15.9)	0.636	20 (18.5)	79 (73.1)	0.78 (0.13–4.46)	0.788
		N	2 (1.8)	7 (6.4)			4 (3.7)	5 (4.6)			1 (0.9)	7 (6.4)			2 (1.8)	7 (6.4)		
	*13	Y	12 (11.1)	63 (58.3)	1.32 (0.36–4.82)	0.673	20 (18.5)	54 (50)	0.5 (0.19–1.27)	0.148	14 (12.9)	60 (55.5)	0.69 (0.25–1.86)	0.465	14 (12.9)	59 (54.6)	0.88 (0.31—2.47)	0.818
		N	5 (4.6)	28 (25.9)			16 (14.8)	18 (16.6)			9 (8.3)	25 (23.1)			8 (7.4)	27 (25)		
	*14	Y	15 (13.89)	78 (72.22)	0.69 (0.12–3.82)	0.679	33 (30.5)	65 (60.1)	0.58 (0.11–2.89)	0.513	21 (19.4)	72 (66.6)	1.43 (0.28–7.23)	0.662	21 (19.4)	73 (67.5)	2.51 (0.29–21.55)	0.4
		N	2 (1.85)	13 (12.04)			3 (2.7)	7 (6.4)			2 (1.8)	13 (12)			1 (0.9)	13 (12.)		
	*15	Y	14 (12.9)	83 (76.8)	0.74 (0.13–4.04)	0.728	32 (29.6)	65 (60.1)	0.81 (0.19–3.43)	0.78	20 (18.5)	78 (72.22)	0.8 (0.18–3.51)	0.776	20 (18.5)	77 (71.3)	1.25 (0.24–6.56)	0.786
		N	3 (2.7)	8 (7.4)			4 (3.7)	7 (6.4)			3 (2.78)	7 (6.48)			2 (1.8)	9 (8.3)		
	*16	Y	_				34 (31.4)	62 (57.4)	3.19 (0.59–17.2)	0.177	21 (19.4)	75 (69.4)	1.38 (0.26–7.23)	0.698	20 (18.5)	76 (70.3)	1.27 (0.24–6.61)	0.771
		N					2 (1.8)	10 (9.2)			2 (1.8)	10 (9.2)			2 (1.8)	10 (9.2)		
	*17	Y	_				31 (28.7)	68 (62.9)	0.56 (0.11–2.72)	0.478	21 (19.4)	78 (72.2)	1.02 (0.18–5.76)	0.975	20 (18.5)	79 (73.1)	2.25 (0.25–20.06)	0.466
		N					5 (4.63)	4 (3.7)			2 (1.8)	7 (6.4)			2 (1.8)	7 (6.4)		
	*18	Y	_				_				_				_			
		N																
DQ	*02	Y	14 (13)	71 (65.7)	1.66 (0.33–8.35)	0.536	28 (25.9)	57 (52.7)	0.86 (0.28–2.65)	0.799	18 (16.6)	67 (62)	0.89 (0.27–2.88)	0.854	16 (14.8)	69 (63.8)	0.75 (0.23–2.44)	0.644
		N	3 (2.8)	20 (18.52)			8 (7.4)	15 (13.8)			5 (4.6)	18 (16.6)			6 (5.5)	17 (15.7)		
	*04	Y	16 (14.8)	73 (67.6)	2.42 (0.28–20.5)	0.416	31 (28.7)	57 (52.7)	1.03 (0.30–3.48)	0.952	_				19 (17.5)	70 (64.8)	1.14 (0.29–4.53)	0.845
		N	1 (0.9)	18 (16.7)			5 (4.6)	14 (12.9)							3 (2.7)	16 (14.1)		
	*05	Y	13 (12)	64 (59.3)	1.12 (0.29–4.3)	0.866	27 (25)	50 (46.3)	2.01 (0.68–5.94)	0.204	17 (15.7)	67 (62.)	0.87 (0.27–2.74)	0.818	13 (12)	64 (59.2)	0.51 (0.18–1.43)	0.204
		N	4 (3.7)	27 (25)			9 (8.3)	22 (20.3)			6 (5.5)	18 (16.6)			9 (8.3)	22 (20.3)		
	*06	Y	9 (8.3)	60 (55.6)	0.82 (0.25–2.67)	0.744	21 (19.4)	48 (44.4)	0.83 (0.33–2.08)	0.7	11 (10.1)	56 (51.8)	0.52 (0.2–1.37)	0.189	16 (14.8)	53 (49)	1.76 (0.6–5.16)	0.298
		N	8 (7.4)	31 (28.7)			15 (13.8)	24 (22.2)			12 (11.1)	29 (26.8)			6 (5.5)	33 (30.5)		
	*07	Y	**7 (6.5)**	**56 (51.8)**	**0.24 (0.06–0.89)**	**0.034**	20 (18.5)	44 (40.7)	0.5 (0.19–1.3)	0.158	12 (11.1)	50 (46.3)	0.62 (0.23–1.67)	0.351	12 (11.1)	51 (47.2)	0.63 (0.23–1.72)	0.372
		N	**10 (9.3)**	**35 (32.4)**			16 (14.8)	28 (25.9)			11 (10.1)	35 (32.4)			10 (9.2)	35 (32.4)		
	*08	Y	15 (13.9)	67 (62)	2.5 (0.5–12.4)	0.26	29 (26.8)	54 (50)	1.56 (0.54–4.51)	0.408	18 (16.6)	64 (59.2)	1.35 (0.43–4.21)	0.602	19 (17.5)	63 (58.3)	2.41 (0.64–9.1)	0.193
		N	2 (1.8)	24 (22.2)			7 (6.4)	18 (16.6)			5 (4.6)	21 (19.4)			3 (2.7)	23 (21.3)		
	*09	Y	14 (12.9)	83 (76.8)	0.23 (0.04–1.27)	0.094	34 (31.4)	63 (58.3)	1.29 (0.23–7.27)	0.768	21 (19.4)	76 (70.3)	0.92 (0.16–5.06)	0.927	20 (18.5)	77 (71.3)	0.84 (0.15–4.57)	0.842
		N	3 (2.8)	8 (7.4)			2 (1.8)	9 (8.3)			2 (1.8)	9 (8.3)			2 (1.8)	9 (8.3)		

CSP = circumsporozoite protein; EBA = erythrocyte binding antigen; HLA = human leukocyte antigen; LSA-1 = liver stage antigen-1; MSP-3 = merozoite surface protein-3; Y = yes (presence of an HLA allele); N = no (absence of an HLA allele); R = responder; NR = nonresponder; N = number of individuals; OR = odds ratio; CI = confidence interval Bold values shows the significant results.

block the effector mechanisms. The binding of the Fc portions of IgG1 and IgG3 cytophilic antibodies to Fc receptors on phagocytic cells triggers a range of effector functions, such as phagocytosis, the production of cytokines and chemokines, cytotoxicity, and the generation of reactive oxygen and nitrogen species.[62] However, the contribution of parasite-reactive IgG2 antibodies to protection against clinical malaria is suggested in some epidemiological settings.[61,63,64] By contrast, several studies have suggested that IgG4 antibodies most likely do not protect against the disease.[65–68] In Brazil, an analysis of the antibody isotypes specific for several *P. falciparum* proteins revealed that all four IgG subclasses are present and, for some proteins, such as the N-terminal region of the P126 protein, individuals with higher levels of the anti-Nt47 cytophilic IgG antibody had significantly lower parasitemia levels.[10] In the present work, the IgG1, IgG2, IgG3, and IgG4 subclasses against LSA-1, EBA, CSP, and MSP-3 were observed.

The results of the present study, which show different IgG subclass profiles in response to the four studied antigens, may be related to the antigen properties because it has been suggested that the characteristics of antigens themselves influence class switching in B cells.[69–71] The results show that CSP induced mainly cytophilic IgG1 and IgG3 antibodies. Similar data were obtained by Oluwasogo and coworkers, which confirmed the prevalence of the cytophilic IgG1 and IgG3 antibodies over the noncytophilic IgG2 and IgG4 subclasses specific to *P. falciparum* CSP in individuals living in Nigeria.[72] Furthermore, the results presented here showed that the levels of IgG3 antibodies against CSP showed a positive correlation with age. Significant age-related increases in IgG3 antibodies to CSP have also been reported in areas of stable malaria transmission, but none were identified in unstable malaria transmission where IgG3 responses to CSP declined with age.[73]

Here, the IgG1 and IgG4 isotypes were preferentially induced by the LSA-1 antigen. In a study performed in Kenya, John and others reported different data: most antibodies against LSA-1 belonged to the IgG1 and IgG3 subclasses, although IgG2 was observed at lower frequencies. However, the IgG4 isotype was not found.[44,74,75] The difference observed between that study and ours may be a result of the cumulative exposure to antigens, which varies according to the intensity of malaria transmission, because IgG1/IgG3 class switching is independently affected by the cumulative exposure to the antigen.[71]

Studies performed by independent research groups have demonstrated the dominance of the MSP-3-specific IgG1 and IgG3 antibodies in different settings.[60,76,77] In our study, MSP-3 preferentially induced an IgG1 isotype, but MSP-3-specific IgG3 antibodies were infrequent. Interestingly, a relatively high frequency of individuals presenting IgG4 antibodies was observed. The high frequency and levels of IgG4 may be related to helminthic infections; it has been suggested that some allergens or helminths are known to induce IgG4 expression and stimulate both IgG4 and IgE induction.[78] However, except for LSA-1, both the frequency and the levels of IgG4 to other studied antigens were low.

In the present study, we noted that EBA mainly induced an IgG1 antibody response, and the levels of this IgG1 antibody response showed a positive correlation with age, consistent with previous reports showing that this *P. falciparum* protein predominantly induced the IgG1 subclass and that the levels of IgG1 against EBA correlated positively with age.[79,80] However, our results differ from those described by others who reported a predominance of the IgG1 and IgG3 subclasses in children living in malaria-endemic areas of Gabon and Mozambique.[15,79]

The HLA class II molecules, originally called immune response genes, play an essential role in stimulating the immune response by binding and presenting antigen peptides to CD4+ T lymphocytes. Differences in HLA binding affinities can result in a decreased binding and an inefficient presentation of peptides to CD4+ T lymphocytes, leading to a decreased cytokine production by CD4+ T lymphocytes and, consequently, a decreased production of antibodies by B lymphocytes, because B lymphocytes must interact with CD4+ T lymphocytes to be activated. Therefore, HLA allelic forms can influence the host capacity to mount both a naturally acquired and an artificially induced antiplasmodial humoral immune response.[18,19,81–89] In view of these data, we hypothesized the possible involvement of HLA class II molecules in the modulation of the antibody specificity profiles induced by *P. falciparum* antigens in the exposed group. In our study, the analysis of the presence of the HLA-DRB1* and HLA-DQB1* allelic groups and the antibody response to CSP, LSA-1, and MSP-3 did not show any association. However, using a multiple logistic regression analysis, corrected for the time of residence in a malaria-endemic area and the number of previous malaria attacks, we observed that HLA-DRB1*11 and HLA-DQB1*7 had the lowest odds ratios for responding to EBA-175. Considering the importance of anti-EBA-175 antibodies in the development of antiparasite immunity and its possible inclusion in a subunit vaccine, additional studies should focus on the evaluation of the genetic restriction of the anti-EBA-175 humoral response.

In conclusion, the results from the present study show that the CSP, LSA-1, EBA, and MSP-3 antigens evoke an antibody response, particularly of the IgG1 subclass, in a high percentage of individuals naturally exposed to *P. falciparum* infections. The results also show that the anti-EBA IgG antibody response appears to be modulated by HLA class II antigens. Given the increasing focus on the use of subunit malaria vaccines, evaluating the influence of class II allele frequencies in ethnically diverse populations may be important before vaccine trials are conducted among people naturally exposed to malaria parasites.

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Authors' addresses: Lilian Rose Pratt-Riccio, Evelyn Kety Pratt Riccio, and Cláudio Tadeu Daniel-Ribeiro, Laboratório de Pesquisa em Malária, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil, E-mails: riccio@ioc.fiocruz.br, ericcio@ioc.fiocruz.br, and ctdr@uol.

com.br. Daiana De Souza Perce-Da-Silva and Dalma Maria Banic, Laboratório de Imunologia Clínica, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil, E-mails: daiana@ioc.fiocruz.br and banic@ioc.fiocruz.br. Josué Da Costa Lima-Junior and Joseli Oliveira-Ferreira, Laboratório de Imunoparasitologia, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil, E-mails: josue@ioc.fiocruz.br and lila@ioc.fiocruz.br. Marcelo Ribeiro-Alves, Laboratório de Pesquisa Clínica em DST/AIDS, Instituto Nacional de Infectologia Evandro Chagas, Fiocruz, Rio de Janeiro, Brazil, E-mail: mribalves1973@gmail.com. Fátima Santos, LACEN, Rondônia, Brazil, E-mail: fatsantosro@gmail.com. Mércia Arruda, Departamento de Imunologia, Centro de Pesquisas Aggeu Magalhães, Fiocruz, Recife, Brazil, E-mail: merciaarruda@gmail.com. Daniel Camus, Service de Parasitologie-Mycologie, Faculte de Médecine, Lille, France, E-mail: daniel.camus@pasteur-lille.fr. Pierre Druilhe, VAC4ALL, Paris, France, E-mail: druilhe@vac4all.org.

REFERENCES

1. World Health Organization, 2016. *World Malaria Report (2016).* Available at: http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/.
2. Langhorne J, Ndungu FM, Sponaas AM, Marsh K, 2008. Immunity to malaria: more questions than answers. *Nat Immunol 9:* 725–732.
3. White MT, Griffin JT, Akpogheneta O, Conway DJ, Koram KA, Riley EM, Ghani AC, 2014. Dynamics of the antibody response to *Plasmodium falciparum* infection in African children. *J Infect Dis 210:* 1115–1122.
4. McGregor IA, 1964. The passive transfer of human malarial immunity. *Am J Trop Med Hyg 13:* 237–239.
5. Bouharoun-Tayoun H, Attanath P, Sabchareon A, Chongsuphajaisiddhi T, Druilhe P, 1990. Antibodies that protect humans against *Plasmodium falciparum* blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes. *J Exp Med 172:* 1633–1641.
6. Bouharoun-Tayoun HP, Druilhe P, 1992. *P. falciparum* malaria: evidence for an isotype imbalance may be responsible for the delayed acquisition of protective immunity. *Infect Immun 60:* 1473–1481.
7. Luty AJ, Mayombo J, Lekoulou F, Mshana R, 1994. Immunologic responses to soluble exoantigens of *Plasmodium falciparum* in Gabonese children exposed to continuous intense infection. *Am J Trop Med Hyg 51:* 720–729.
8. Bouharoun-Tayoun H, Oeuvray C, Lunel F, Druilhe P, 1995. Mechanisms underlying the monocyte-mediated antibody-dependent killing of *Plasmodium falciparum* asexual blood stages. *J Exp Med 182:* 409–418.
9. Aribot G, Rogier C, Sarthou JL, Trape JF, Balde AT, Druilhe P, Roussilhon C, 1996. Pattern of immunoglobulin isotype response to *Plasmodium falciparum* blood-stage antigens in individuals living in a holoendemic area of Senegal (Dielmo, west Africa). *Am J Trop Med Hyg 54:* 449–457.
10. Banic DM, Oliveira-Ferreira J, Pratt-Riccio LR, Conseil V, Gonçalves D, Fialho RR, Gras-Masse H, Daniel-Ribeiro CT, Camus D, 1998. Immune response and lack of immune response to *Plasmodium falciparum* P126 antigen and its amino-terminal repeat in malaria-infected humans. *Am J Trop Med Hyg 58:* 768–774.
11. Taylor RR, Allen SJ, Greenwood BM, Riley EM, 1998. IgG3 antibodies to *Plasmodium falciparum* merozoite surface protein 2 (MSP2): increasing prevalence with age and association with clinical immunity to malaria. *Am J Trop Med Hyg 58:* 406–413.
12. Ndungu FM, Bull PC, Ross A, Lowe BS, Kabiru E, Marsh K, 2002. Naturally acquired immunoglobulin (Ig)G subclass antibodies to crude asexual *Plasmodium falciparum* lysates: evidence for association with protection for IgG1 and disease for IgG2. *Parasite Immunol 24:* 77–82.
13. Soe S, Theisen M, Roussilhon C, Aye KS, Druilhe P, 2004. Association between protection against clinical malaria and antibodies to merozoite surface antigens in an area of hyperendemicity in Myanmar: complementarity between responses to merozoite surface protein 3 and the 220-kilodalton glutamate-rich protein. *Infect Immun 72:* 247–252.
14. Lima-Junior JC, et al., 2011. B cell epitope mapping and characterization of naturally acquired antibodies to the *Plasmodium vivax* merozoite surface protein-3α (PvMSP-3α) in malaria exposed individuals from Brazilian Amazon. *Vaccine 29:* 1801–1811.
15. Dobaño C, et al., 2012. Age-dependent IgG subclass responses to *Plasmodium falciparum* EBA-175 are differentially associated with incidence of malaria in Mozambican children. *Clin Vaccine Immunol 19:* 157–166.
16. Balogun HA, Awah N, Nilsson S, Rogier C, Trape JF, Chen Q, Roussilhon C, Berzins K, 2013. Pattern of antibodies to the Duffy binding like domain of *Plasmodium falciparum* antigen Pf332 in Senegalese individuals. *Acta Trop 130:* 80–87.
17. Osier FH, et al., 2014. Opsonic phagocytosis of *Plasmodium falciparum* merozoites: mechanism in human immunity and a correlate of protection against malaria. *BMC Med 12:* 108.
18. Banic DM, Goldberg AC, Pratt-Riccio LR, Oliveira-Ferreira J, Santos F, Gras-Masse H, Camus D, Kalil J, Daniel-Ribeiro CT, 2002. Human leukocyte antigen class II control of the immune response to p126-derived amino terminal peptide from *Plasmodium falciparum*. *Am J Trop Med Hyg 66:* 509–515.
19. Pratt-Riccio LR, Lima-Junior JC, Carvalho LJ, Theisen M, Espíndola-Mendes EC, Santos F, Oliveira-Ferreira J, Goldberg AC, Daniel-Ribeiro CT, Banic DM, 2005. Antibody response profiles induced by *Plasmodium falciparum* glutamate-rich protein in naturally exposed individuals from a Brazilian area endemic for malaria. *Am J Trop Med Hyg 73:* 1096–1103.
20. Pratt-Riccio LR, et al., 2008. Evaluation of the genetic polymorphism of *Plasmodium falciparum* P126 protein (SERA or SERP) and its influence on naturally acquired specific antibody responses in malaria-infected individuals living in the Brazilian Amazon. *Malar J 7:* 144.
21. Pratt-Riccio LR, et al., 2011. Antibodies against the *Plasmodium falciparum* glutamate-rich protein from naturally exposed individuals living in a Brazilian malaria-endemic area can inhibit in vitro parasite growth. *Mem Inst Oswaldo Cruz 106 (Suppl 1):* 34–43.
22. Riccio EK, Totino PR, Pratt-Riccio LR, Ennes-Vidal V, Soares IS, Rodrigues MM, Souza JM, Daniel-Ribeiro CT, Ferreira-da-Cruz MF, 2013. Cellular and humoral immune responses against the *Plasmodium vivax* MSP-1_{19} malaria vaccine candidate in individuals living in an endemic area in north-eastern Amazon region of Brazil. *Malar J 12:* 326.
23. Oliveira-Ferreira J, Lacerda MV, Brasil P, Ladislau JL, Tauil PL, Daniel-Ribeiro CT, 2010. Malaria in Brazil: an overview. *Malar J 9:* 115.
24. Andrade AL, Martelli CM, Oliveira RM, Arias JR, Zicker F, Pang L, 1995. High prevalence of asymptomatic malaria in gold mining areas in Brazil. *Clin Infect Dis 20:* 475.
25. Alves FP, Durlacher RR, Menezes MJ, Krieger H, Silva LH, Camargo EP, 2002. High prevalence of asymptomatic *Plasmodium vivax* and *Plasmodium falciparum* infections in native Amazonian populations. *Am J Trop Med Hyg 66:* 641–648.
26. Tada MS, Ferreira RG, Katsuragawa TH, Martha RC, Costa JD, Albrecht L, Wunderlich G, Silva LH, 2012. Asymptomatic infection with *Plasmodium falciparum* and *Plasmodium vivax* in the Brazilian Amazon Basin: to treat or not to treat? *Mem Inst Oswaldo Cruz 107:* 621–629.
27. Gomes LR, et al., 2013. Asymptomatic infection in individuals from the municipality of Barcelos (Brazilian Amazon) is not associated with the anti-*Plasmodium falciparum* glycosylphosphatidyinositol antibody response. *Mem Inst Oswaldo Cruz 108:* 796–800.
28. Mendonça VR, Souza LC, Garcia GC, Magalhães BM, Lacerda MV, Andrade BB, Gonçalves MS, Barral-Netto M, 2014. DDX39B (BAT1), TNF and IL6 gene polymorphisms and association with clinical outcomes of patients with *Plasmodium vivax* malaria. *Malar J 13:* 278.
29. Shute GT, 1988. The microscpopic diagnosis of malaria. Wernsdorfer WH, McGregor SI, eds. *Malaria: Principles and Practice of Malariology.* New York, NY: Churchill Livingstone, 781–814.
30. Snounou G, 1996. Detection and identification of the four malaria parasite species infecting humans by PCR amplification. *Methods Mol Biol 50:* 263–291.

31. Gausepohl H, Boulin C, Kraft M, Frank RW, 1996. Automated multiple peptide synthesis. *Pept Res 5:* 315–320.
32. Oliveira-Ferreira J, Pratt-Riccio LR, Arruda M, Santos F, Daniel-Ribeiro CT, Goldberg AC, Banic DM, 2004. HLA class II and antibody responses to circumsporozoite protein repeats of *P. vivax* (VK210, VK247 and *P. vivax*-like) in individuals naturally exposed to malaria. *Acta Trop 92:* 63–69.
33. Olerup O, Zetterquist H, 1992. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens 39:* 225–235.
34. Bunce M, O'Neil CM, Barnardo MCNM, Krausa P, Browning MJ, Morris PJ, Welsh KI, 1995. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 and DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens 46:* 355–367.
35. Baur MP, Danilovs JA, 1980. Population analysis of HLA-A, B, C, DR and other genetic markers. Terasaki PI, ed. *Histocompatibility Testing*. Los Angeles, CA: UCLA Tissue Typing Laboratory, 955–1210.
36. Dodoo D, et al., 2011. Measuring naturally acquired immune responses to candidate malaria vaccine antigens in Ghanaian adults. *Malar J 10:* 168.
37. Camargo LM, Noronha E, Salcedo JM, Dutra AP, Krieger H, Pereira da Silva LH, Camargo EP, 1999. The epidemiology of malaria in Rondonia (western Amazon region, Brazil): study of a riverine population. *Acta Trop 72:* 1–11.
38. Tada MS, Marques RP, Mesquita E, Dalla Martha RC, Rodrigues JA, Costa JDN, Pepelascov RR, Katsuragawa TH, Pereira-da-Silva LH, 2007. Urban malaria in the Brazilan western Amazon Region. I. High prevalence of asymptomatic carriers in an urban riverside district is associated with a high level of clinical malaria. *Mem Inst Oswaldo Cruz 102:* 263–269.
39. Costa JD, Zanchi FB, Rodrigues FL, Honda ER, Katsuragawa TH, Pereira DB, Taborda RL, Tada MS, Ferreira RG, Pereira-da-Silva LH, 2013. Cross-reactive anti-PfCLAG9 antibodies in the sera of asymptomatic parasite carriers of *Plasmodium vivax*. *Mem Inst Oswaldo Cruz 108:* 98–105.
40. Fratus AS, Cabral FJ, Fotoran WL, Medeiros MM, Carlos BC, Martha R, da Silva LH, Lopes SC, Costa FT, Wunderlich G, 2014. Antibody recognition of *Plasmodium falciparum* infected red blood cells by symptomatic and asymptomatic individuals in the Brazilian Amazon. *Mem Inst Oswaldo Cruz 109:* 598–601.
41. Egan AF, Morris J, Barnish G, Allen S, Greenwood BM, Kaslow DC, Holder AA, Riley EM, 1996. Clinical immunity to *Plasmodium falciparum* malaria is associated with serum antibodies to the 19-kDa C-terminal fragment of the merozoite surface antigen, PfMSP-1. *J Infect Dis 173:* 765–769.
42. Branch OH, Udhayakumar V, Hightower AW, Oloo AJ, Hawley WA, Nahlen BL, Bloland PB, Kaslow DC, Lal AA, 1998. A longitudinal investigation of IgG and IgM antibody responses to the merozoite surface protein-1 19-kiloDalton domain of *Plasmodium falciparum* in pregnant women and infants: associations with febrile illness, parasitemia, and anemia. *Am J Trop Med Hyg 58:* 211–219.
43. Roussilhon C, Oeuvray C, Muller-Graf C, Tall A, Rogier C, Trape JF, Theisen M, Balde A, Perignon JL, Druilhe P, 2007. Long-term clinical protection from falciparum malaria is strongly associated with IgG3 antibodies to merozoite surface protein 3. *PLoS Med 4:* e320.
44. John CC, Tande AJ, Moormann AM, Sumba PO, Lanar DE, Min XM, Kazura JW, 2008. Antibodies to pre-erythrocytic *Plasmodium falciparum* antigens and risk of clinical malaria in Kenyan children. *J Infect Dis 197:* 519–526.
45. Nebie I, et al., 2008. Humoral responses to *Plasmodium falciparum* blood-stage antigens and association with incidence of clinicalmalaria in children living in an area of seasonal malaria transmission in Burkina Faso, West Africa. *Infect Immun 76:* 759–766.
46. Osier FH, et al., 2008. Breadth and magnitude of antibody responses to multiple *Plasmodium falciparum* merozoite antigens are associated with protection from clinical malaria. *Infect Immun 76:* 2240–2248.
47. Ford L, Lobo CA, Rodriguez M, Zalis MG, Machado RL, Rossit AR, Cavasini CE, Couto AA, Enyong PA, Lustigman S, 2007. Differential antibody responses to *Plasmodium falciparum* invasion ligand proteins in individuals living inmalaria-endemic areas in Brazil and Cameroon. *Am J Trop Med Hyg 77:* 977–983.
48. Sarr JB, et al., 2012. Differential acquisition of human antibody responses to *Plasmodium falciparum* according to intensity of exposure to *Anopheles* bites. *Trans R Soc Trop Med Hyg 106:* 460–467.
49. Okenu DM, Riley EM, Bickle QD, Agomo PU, Barbosa A, Daugherty JR, Lanar DE, Conway DJ, 2000. Analysis of human antibodies to erythrocyte binding antigen 175 of *Plasmodium falciparum*. *Infect Immun 68:* 5559–5566.
50. Richards JS, et al., 2010. Association between naturally acquired antibodies to erythrocyte-binding antigens of *Plasmodium falciparum* and protection from malaria and high-density parasitemia. *Clin Infect Dis 51:* e50–e60.
51. Villasis E, Lopez-Perez M, Torres K, Gamboa D, Neyra V, Bendezu J, Tricoche N, Lobo C, Vinetz JM, Lustigman S, 2012. Anti-*Plasmodium falciparum* invasion ligand antibodies in a low malaria transmission region, Loreto, Peru. *Malar J 11:* 361.
52. Nhabomba AJ, et al., 2014. Impact of age of first exposure to *Plasmodium falciparum* on responses to malaria in children: a randomized, controlled trial in Mozanbique. *Malar J 13:* 121.
53. John CC, Moormann AM, Pregibon DC, Sumba PO, McHugh MM, Narum DL, Lanar DE, Schluchter MD, Kazura JW, 2005. Correlation of high levels of antibodies to multiple pre-erythrocytic *Plasmodium falciparum* antigens and protection from infection. *Am J Trop Med Hyg 73:* 222–228.
54. Arama C, et al., 2015. Genetic resistance to malaria is associated with greater enhancement of immunoglobulin (Ig)M than IgG responses to a broad array of *Plasmodium falciparum* antigens. *Open Forum Infect Dis 2:* ofv118.
55. Riley EM, Allen SJ, Wheeler JG, Blackman MJ, Bennett S, Takacs B, Schönfeld HJ, Holder AA, Greenwood BM, 1992. Naturally acquired cellular and humoral immune responses to the major merozoite surface antigen (PfMSP1) of *Plasmodium falciparum* are associated with reduced malaria morbidity. *Parasite Immunol 14:* 321–337.
56. Al-Yaman F, Genton B, Anders RF, Falk M, Triglia T, Lewis D, Hii J, Beck HP, Alpers MP, 1994. Relationship between humoral response to *Plasmodium falciparum* merozoite surface antigen-2 and malaria morbidity in a highly endemic area of Papua New Guinea. *Am J Trop Med Hyg 51:* 593–602.
57. Cavanagh DR, Dodoo D, Hviid L, Kurtzhals JA, Theander TG, Akanmori BD, Polley S, Conway DJ, Koram K, McBride SJ, 2004. Antibodies to the N-terminal block 2 of *Plasmodium falciparum* merozoite surface protein 1 are associated with protection against clinical malaria. *Infect Immun 72:* 6492–6502.
58. Metzger WG, Okenu DM, Cavanagh DR, Robinson JV, Bojang KA, Weiss HA, McBride JS, Greenwood BM, Conway DJ, 2003. Serum IgG3 to the *Plasmodium falciparum* merozoite surface protein 2 is strongly associated with reduced prospective risk of malaria. *Parasite Immunol 25:* 307–312.
59. Polley SD, Conway DJ, Cavanagh DR, McBride JS, Lowe BS, Williams TN, Mwangi TW, Marsh K, 2006. High levels of serum antibodies to merozoite surface protein 2 of *Plasmodium falciparum* are associated with reduced risk of clinical malaria in coastal Kenya. *Vaccine 24:* 4233–4246.
60. Stanisic DI, et al., 2009. Immunoglobulin-G subclass-specific responses against *Plasmodium falciparum* merozoite antigens are associated with control of parasitemia and protection from symptomatic illness. *Infect Immun 77:* 1165–1174.
61. Cherif MK, et al., 2015. Is Fc gamma receptor IIA (FcγRIIA) polymorphism associated with clinical malaria and *Plasmodium falciparum* specific antibody levels in children from Burkina Faso? *Acta Trop 142:* 41–46.
62. Ortega E, Soto-Cruz I, 2007. Early biochemical events in leukocyte activation through receptors for IgG. *Signal Transduct 7:* 415–426.
63. Aucan C, Traoré Y, Tall F, Nacro B, Traoré-Leroux T, Fumoux F, Rihet P, 2000. Infect High immunoglobulin G2 (IgG2) and low IgG4 levels are associated with human resistance to *Plasmodium falciparum* malaria. *Immun. 68:* 1252–1258.

64. Nasr A, Iriemenam NC, Troye-Blomberg M, Giha HA, Balogun HA, Osman OF, Montgomery SM, ElGhazali G, Berzins K, 2007. Fc gamma receptor IIa (CD32) polymorphism and antibody responses to asexual blood-stage antigens of *Plasmodium falciparum* malaria in Sudanese patients. *Scand J Immunol 66:* 87–96.
65. Dubois B, Deloron P, Astagneau P, Chougnet C, Lepers JP, 1993. Isotypic analysis of *Plasmodium falciparum*-specific antibodies and their relation to protection in Madagascar. *Infect Immun 61:* 4498–4500.
66. Lucchi NW, et al., 2008. Antibody responses to the merozoite surface protein-1 complex in cerebral malaria patients in India. *Malar J 7:* 121.
67. Sarthou JL, Angel G, Aribot G, Rogier C, Dieye A, Toure Balde A, Diatta B, Seignot P, Roussilhon P, 1997. Prognostic value of anti-*Plasmodium falciparum*-specific immunoglobulin G3, cytokines, and their soluble receptors in West African patients with severe malaria. *Infect Immun 65:* 3271–3276.
68. Tangteerawatana P, Krudsood S, Chalermrut K, Looareesuwan S, Khusmith S, 2001. Natural human IgG subclass antibodies to *Plasmodium falciparum* blood stage antigens and their relation to malaria resistance in an endemic area of Thailand. *Southeast Asian J Trop Med Public Health 32:* 247–254.
69. Snapper CM, Finkelman FD, 1999. Immunoglobulin class switching. Paul WE, ed., *Fundamental Immunology*, 4th edition. Philadelphia, PA: Lippincott-Raven, 831–861.
70. Stavnezer J, 1996. Immunoglobulin class switching. *Curr Opin Immunol 8:* 199–205.
71. Tongren JE, et al., 2006. Target antigen, age, and duration of antigen exposure independently regulate immunoglobulin G subclass switching in malaria. *Infect Immun 74:* 257–264.
72. Oluwasogo OA, Ebenezer OO, Chiaka A, 2012. Evaluation of host humoral antibody production against *Plasmodium falciparum* recombinant circumsporozoite antigen in Nigerian children. *J Vector Borne Dis 49:* 151–156.
73. Noland GS, Jansen P, Vulule JM, Park GS, Ondigo BN, Kazura JW, Moormann AM, John CC, 2015. Effect of transmission intensity and age on subclass antibody responses to *Plasmodium falciparum* pre-erythrocytic and blood-stage antigens. *Acta Trop 142:* 47–56.
74. John CC, Ouma JH, Sumba PO, Hollingdale MR, Kazura JW, King CL, 2002. Lymphocyte proliferation and antibody responses to *Plasmodium falciparum* liver-stage antigen-1 in a highland area of Kenya with seasonal variation in malaria transmission. *Am J Trop Med Hyg 66:* 372–378.
75. John CC, Zickafoose JS, Sumba PO, King CL, Kazura JW, 2003. Antibodies to the *Plasmodium falciparum* antigens circumsporozoite protein, thrombospondin-related adhesive protein, and liver-stage antigen 1 vary by ages of subjects and by season in a highland area of Kenya. *Infect Immun 71:* 4320–4325.
76. Courtin D, et al., 2009. The quantity and quality of African children's IgG responses to merozoite surface antigens reflect protection against *Plasmodium falciparum* malaria. *PLoS One 4:* e7590.
77. Mamo H, Esen M, Ajua A, Theisen M, Mordmüller B, Petros B, 2013. Humoral immune response to *Plasmodium falciparum* vaccine candidate GMZ2 and its components in populations naturally exposed to seasonal malaria in Ethiopia. *Malar J 12:* 51.
78. Garraud O, Nkenfou C, Bradley JE, Perler FB, Nutman TB, 1995. Identification of recombinant filarial proteins capable of inducing polyclonal and antigen-specific IgE and IgG4 antibodies. *J Immunol 155:* 1316–1325.
79. Toure FS, Deloron P, Migot-Nabias F, 2006. Analysis of human antibodies to erythrocyte binding antigen 175 peptide 4 of *Plasmodium falciparum*. *Clin Med Res 4:* 1–6.
80. Mewono L, Matondo-Maya DW, Matsiegui PB, Agnandji ST, Kendjo E, Barondi F, Issifou S, Kremsner PG, Mavoungou E, 2008. Interleukin-21 is associated with IgG1 and IgG3 antibodies to erythrocyte-binding antigen-175 peptide 4 of *Plasmodium falciparum* in Gabonese children with acute falciparum malaria. *Eur Cytokine Netw 19:* 30–36.
81. Patarroyo ME, et al., 1991. Genetic control of the immune response to a synthetic vaccine against *Plasmodium falciparum*. *Parasite Immunol 13:* 509–516.
82. Beck HP, Felger I, Genton B, Alexander N, al-Yaman F, Anders RF, Alpers M, 1995. Humoral and cell-mediated immunity to the *Plasmodium falciparum* ring-infected erythrocyte surface antigen in an adult population exposed to highly endemic malaria. *Infect Immun 63:* 596–600.
83. Stephens HA, Brown AE, Chandanayingyong D, Webster HK, Sirikong M, Longta P, Vangseratthana R, Gordon DM, Lekmak S, Rungruang E, 1995. The presence of the HLA class II allele DPB1*0501 in ethnic Thais correlates with an enhanced vaccine-induced antibody response to a malaria sporozoite antigen. *Eur J Immunol 25:* 3142–3147.
84. Nardin EH, Oliveira GA, Calvo-Calle JM, Castro ZR, Nussenzweig RS, Schmeckpeper B, Hall BF, Diggs C, Bodison S, Edelman R, 2000. Synthetic malaria peptide vaccine elicits high levels of antibodies in vaccinees of defined HLA genotypes. *J Infect Dis 182:* 1486–1496.
85. Johnson AH, et al., 2004. Human leukocyte antigen class II alleles influence levels of antibodies to the *Plasmodium falciparum* asexual-stage apical membrane antigen 1 but not to merozoite surface antigen 2 and merozoite surface protein 1. *Infect Immun 72:* 2762–2771.
86. Zhang Q, Xue X, Xu X, Wang C, Chang W, Pan W, 2009. Influence of HLA-DRB1 alleles on antibody responses to PfCP-2.9-immunized and naturally infected individuals. *J Clin Immunol 29:* 454–460.
87. Storti-Melo LM, et al., 2012. Influence of HLA-DRB-1 alleles on the production of antibody against CSP, MSP-1, AMA-1, and DBP in Brazilian individuals naturally infected with *Plasmodium vivax*. *Acta Trop 121:* 152–155.
88. Lima-Junior JC, et al., 2012. Influence of HLA-DRB1 and HLA-DQB1 alleles on IgG antibody response to the *P. vivax* MSP-1, MSP-3α and MSP-9 in individuals from Brazilian endemic area. *PLoS One 7:* e36419.
89. Lima-Junior J, Pratt-Riccio LR, 2016. Major histocompatibility complex and malaria: focus on *Plasmodium vivax* infection. *Front Immunol 7:* 13.