Abstract. The humoral immune response against synthetic peptides of two *Plasmodium falciparum* blood-stage antigens, Pf155/ring-infected erythrocyte surface antigen (RESA) (EENV), and Pf332 (SVTEEIAEEDK), in individuals belonging to three sympatric ethnic groups (Mossi, Rimaibe, and Fulani) living in the same conditions of hyperendemic transmission in a Sudan savanna area northeast of Ouagadougou, Burkina Faso were examined. The Mossi and Rimaibe are Sudanese Negroid populations with a long tradition of sedentary farming, while the Fulani are nomadic pastoralists partly settled and characterized by non-Negroid features of possible Caucasian origin. A total of 764 subjects (311 Mossi, 273 Rimaibe, and 180 Fulani) were tested. A lower *P. falciparum* prevalence was observed in the Fulani of all age groups. The serologic results clearly indicate the existence of interethnic differences in the capacity to respond to these two *P. falciparum* antigens. The Mossi and Rimaibe showed similar responses, whereas the Fulani displayed consistently higher prevalences and levels of antibodies against both epitopes tested. The anti-(EENV), and anti-(SVTEEIAEEDK) seroprevalences were 29.9% and 38.9% in Mossi, 29.7% and 39.2% in Rimaibe, 86.1% and 76.1% in Fulani (all \( P \) values of Fulani-Mossi and Fulani-Rimaibe comparisons \( \approx 0.001 \)). Anti-RESA and anti-Pf332 antibody levels were approximately 65% (\( P < 0.001 \)) and 45% (\( P < 0.001 \)), respectively, higher in seropositive Fulani than in seropositive Mossi and Rimaibe, who showed very similar values. The observed differences cannot be explained in terms of interethnic heterogeneity of malaria exposure since these communities have lived in the same area for more than 30 years and the *P. falciparum* inoculation rate, measured during two consecutive years, was substantially uniform for the three ethnic groups. The possibility of remarkable heterogeneities in the capacity to mount immune responses against *P. falciparum* antigens among populations with different genetic backgrounds must be taken into account in the development of anti-malaria vaccines.

The comparison of the response to *Plasmodium falciparum* antigens among populations with different genetic backgrounds exposed to high and uniform transmission of the same parasite strains is one of the possible approaches to detect genetically based heterogeneities in the immune response to malaria. This approach has been recently applied to the study of the anti-circumsporozoite protein (CSP), anti-thrombospondin-related anonymous protein (TRAP), and anti-merozoite surface antigen-1 (MSA-1) immune responses\(^1\) in three sympatric West African ethnic groups, the Fulani, Mossi, and Rimaibe. The Fulani genetic/cultural background is well differentiated from that of the Mossi and Rimaibe, since the former are nomadic pastoralists with non-Negroid features of possible Caucasian origin,\(^3\) while the latter are Sudanese Negroid populations with a long tradition of sedentary farming in sub-Saharan savannas.\(^4\) Closer to the Mossi in terms of ethnic origin, the Rimaibe have adopted most of the sociocultural habits of the Fulani, having been their slaves. The increasing tendency of the Fulani to settle and converge with the habits of sedentary populations occasionally determines sufficiently stable sympatric and even syntopic conditions that allow the interethnic comparison.

The above mentioned studies clearly showed higher immune reactivity to the three antigens tested in the Fulani as compared with the Mossi and Rimaibe. Further tests were carried out with the Pf155/ring-infected erythrocyte surface antigen (RESA) and Pf332. The results are presented in this report.

The Pf155/RESA and the Pf332 are two antigens of the asexual erythrocytic stages of *P. falciparum*. The Pf155/RESA is one of the candidate antigens for inclusion in a vaccine against the asexual blood stages of the parasite. The antigen is a nonglycosylated polypeptide of 155 kD that is expressed on the interior side of the erythrocyte membrane shortly after merozoite invasion, where it can be detected by a modified indirect immunofluorescence assay.\(^5\) The antigen contains two extensive blocks of tandemly repeated amino acid sequences, one in the C-terminus comprising five repeats of the octamer EENVEHDA and about 35 repeats of the tetramer EENV, with a small degenerate area in the end of the molecule. The second repeat region is located in the center of the molecule comprising seven 11-mer repeats with the consensus sequence DDEHVEEPTVA.\(^6\) The Pf155/RESA has been thoroughly analyzed for the presence of both B and T cell epitopes. Immunodominant B cell epitopes are located within the C-terminal repeats.\(^7,8\) Several seroepidemiologic studies have shown correlations between antibody levels, particularly those reactive with EENV sequence, and reduced parasitemia and malaria morbidity in individuals from various study areas.\(^9-14\) Furthermore, antibodies reactive with Pf155/RESA repeats are very efficient inhibitors of *P. falciparum* merozoite invasion into erythrocytes in vitro.\(^13,15,16\) The Pf332 antigen is expressed on the surface of erythrocytes infected with late stages of *P. falciparum* and appears to be a major target for opsonic antibodies.\(^17\) The antigen contains 11 amino acid repeats rich in glutamic acid. Individuals living in malaria-endemic regions show a high prevalence of seroreactivity with these repeats.\(^18\)

The aim of this study was to evaluate the influence of the human genetic background on the capacity to mount a humoral response against B cell epitopes of Pf155/RESA and Pf332.
MALARIA IMMUNE RESPONSE IN SYMPATRIC ETHNIC GROUPS

MATERIALS AND METHODS

Study area and subjects. A detailed description of the study area has been previously reported. The study was carried out in two rural villages 35 km northeast of Ouagadougou, the capital city of Burkina Faso, in a shrubby savanna of the Mossi plateau (about 300 meters above sea level) belonging to the Sudan-Sahelian ecoclimatic zone (isohyets = 600–900 mm). Intense P. falciparum transmission is recorded during the June–October rainy season, frequently reaching mean sporozoite inoculation rates well above one infective bite/person/night. The main malaria vectors are *Anopheles gambiae*, *An. arabiensis*, and *An. funestus*. The results of extensive entomologic surveys carried out during the 1994 and 1995 main transmission periods (August–November) and in the intervening dry season (March 1995) showed similar *P. falciparum* entomologic inoculation rates for the three ethnic groups.

The study protocol was approved by the Centre National de Lutte contre le Paludisme of the Ministry of Health of Burkina Faso. The samples analyzed were collected after oral informed consent was obtained in August 1994 from a Fulani community living in the village of Barkoundouba and from a Mossi/Rimbaïe community living about 5 km apart in the village of Barkoumbilen. These sedentary communities have lived in the two villages for more than 30 years; before 1964, all the three groups lived in the same village of Barkoumbilen. A total of 764 subjects (311 Mossi, 273 Rimaïbe, and 180 Fulani) of all age classes were included in the study; the mean ± SE ages were 21.5 ± 1.1 years in the Mossi, 18.7 ± 1.0 years in the Rimaïbe, and 20.2 ± 1.3 years in the Fulani.

Blood examination. Thick and thin blood smears were prepared following standard procedures and 100 microscope fields (approximately 20 leukocytes/field at 1,000× = approximately 0.25 μl of blood) of the thick blood smear were examined. The *Plasmodium* species was identified on the thin blood smear.

Serologic tests. Blood samples were collected into sterile tubes containing tripotassium EDTA. Within 3–4 hr after bleeding, the plasma was removed and kept at −20°C until serologic tests were done. The humoral response against the carboxy-terminal B cell epitope of the Pf155/RESA (EENV)6 and against the (SVTEEIAEEDK)2 epitope of the Pf332 antigen was measured by ELISA. Polysorp immunoplates (Nunc, Wiesbaden, Germany) were coated with the RESA-peptide (EENV)6-bovine serum albumin (BSA) or with the Pf332 peptide (SVTEEIAEEDK)2-BSA at a concentration of 5 μg/ml. The wells were then incubated for 2 hr at 37°C with 0.5% BSA in phosphate-buffered saline to prevent nonspecific binding. Samples were diluted 1:200 and tested in duplicate. The plasma were allowed to react for 1 hr at room temperature. For the detection of bound antibodies, goat anti-human immunoglobulin serum conjugated to alkaline phosphatase (S382B; Promega, Leiden, The Netherlands) was added at a dilution of 1:7,500 for 1 hr at room temperature. Bound phosphatase activity was revealed by adding a solution (5 mg/ml) of 4-nitrophenylphosphate in 10% diethanolamine, 10 mM MgCl2. After developing for 60 min, the optical density at 405 nm (OD405) was measured at with a Titertek (Flow Laboratories, McLean, VA) spectrophotometer. The cut-off values for positive reactions were calculated as the mean OD405 + 3 SD of the values obtained from 20 nonexposed, non-immune Italian sera. The blank wells gave a mean ± SD OD405 of 0.069 ± 0.021 (n = 22) and 0.072 ± 0.007 (n = 22) for RESA and Pf332, respectively. The Italian sera gave a mean ± SD OD405 of 0.09 ± 0.02 for Pf155/RESA (EENV)6, and of 0.07 ± 0.05 for the Pf332 peptide (SVTEEIAEEDK)2. The curve relating OD and dilution was linear on a linear-log scale over the range 0.20–1.5 OD405 for both antigens. Equivalent proportions of ethnic subsamples were tested in each plate. Antibody levels were expressed as log10 of the absorbances. To check for interassay variability, a negative control, a blank, and a six-point standard curve obtained by serial dilutions of a highly positive sera were introduced in each plate.

Statistical methods. The Yates’-corrected chi-square test was used for the analysis of parasite rates and seroprevalences, and the Kruskall-Wallis test was used for the comparisons of age means, parasite density, and antibody levels. To calculate the standard errors of prevalences, the following formula was used: √[p(1–p)/N]; for the standard errors of antibody levels, we first transformed the OD405 absorbances into log10 absorbances to normalize the distribution, then we checked that their distribution was compatible with a normal distribution and finally we calculated the standard errors with the usual formula (standard deviation)/√N. Version 5 of the program Epi-Info21 was used.

RESULTS

Parasite rates and density. The results of five consecutive parasitologic/clinical cross-sectional surveys performed in the study area have been previously reported. The parasitologic data of the August 1994 survey, corresponding to the serologic data presented here, are summarized in Figure 1A. A lower *P. falciparum* prevalence was observed in the Fulani of all age groups. The differences were particularly marked in the older age groups. No interethnic differences were recorded in the positive parasite densities and no intraethnic difference between sexes was found.

Humoral immune responses. The total anti-(EENV)6 and anti-(SVTEEIAEEDK)2 mean ± SE seroprevalences were 43.1 ± 1.80% and 47.8 ± 1.81%, respectively. The mean ± SE anti-(EENV)6 and anti-(SVTEEIAEEDK)2 seroprevalences were 29.9 ± 2.6% (93 of 311) and 38.9 ± 2.8% (121 of 311) in the Mossi, 29.7 ± 2.8% (81 of 273) and 39.2 ± 3.0% (107 of 273) in the Rimaïbe, and 86.1 ± 2.6% (155 of 180) and 76.1 ± 3.2% (137 of 180) in the Fulani. The prevalences and levels of antibodies against the (EENV)6 epitope of the Pf155/RESA and against the (SVTEEIAEEDK)2 epitope of the Pf332 analyzed by age and ethnic group are summarized in Figures 1B and 1C. The prevalence and levels of antibodies to the two synthetic peptides increased with age in the three ethnic groups; in all age groups and for both antigens, the Fulani showed higher seroprevalences and higher levels of antibodies than the Mossi and the Rimaïbe, who showed similar values. No intraethnic difference between sexes was recorded and no interethnic associations were found between anti-(EENV)6 and anti-(SVTEEIAEEDK)2 antibodies and *P. falciparum* parasitemia. A strong association within all age groups (P < 0.001
FIGURE 1. Mean ± standard error values by age and ethnic group of *Plasmodium falciparum* parasite rates (A) and anti-Pf155/ring-infected erythrocyte surface antigen (RESA) (EENV), and anti-Pf332 (SVTEEIAEEDK) antibody prevalences (B) and levels (C) by age and ethnic group. The positions of the symbols on the x-axis correspond to the mean age for each of the following age groups: 0–5, 6–10, 11–20, 21–30, and > 30 years. To indicate a significant difference between the Fulani and one or both of the other two groups, half-filled or filled symbols, respectively, are used. Figure 1A is modified from Modiano and others.²

for each ethnic group) between anti-(EENV)₆ and anti-(SVTEEIAEEDK)₂ immune responses was observed.

**DISCUSSION**

The capacity to mount humoral immune responses against epitopes of *P. falciparum* antigens is markedly different in population samples from the sympatric ethnic groups (Fulani, Mossi, and Rimaibe) of the Ouagadougou area in Burkina Faso. For both Pf155/RESA and Pf332 antigens, the responses were much stronger in the non-Negroid Fulani. Even in those more than 30 years of age, the Negroid Mossi and Rimaibe showed lower seroprevalences than those recorded in the 0–5-years-old age group in the Fulani. These results closely correspond to those previously reported¹⁻² on the CSP, TRAP, and MSA-1 antigens and provide a picture of remarkably consistent interethnic differences in the immune response, which cannot be explained by corresponding heterogeneities in parasite exposure. More specifically, the entomologic surveys performed during two consecutive years in our study area² demonstrated similar *P. falciparum* inoculation rates for the three ethnic groups. Moreover, the stronger immune response of the Fulani is associated with lower *P. falciparum* parasite rates and incidence of clinical malaria. None of the cultural characteristics of the Fulani, including their dietary habits, may account for the parasitologic, clinical, and immunologic differences observed, particularly in view of their magnitude and stability. This is further supported by the Rimaibe response to malaria, which diverges from that of the Fulani in spite of important cultural similarities, and closely corresponds to that of the Mossi, in agreement with the anthropologic relationships. The consequent working hypothesis is that we are dealing with the expression of genetic differences between the non-Negroid Fulani and the Negroid Rimaibe and Mossi.

In a longitudinal prospective study of clinical immunity to *P. falciparum* malaria in The Gambia, Riley and others²² observed a significant association between the presence of antibodies to the 3’ repeat region peptide (EENV), of the Pf155/RESA and resistance to clinical malaria. Moreover,
Riley and others\textsuperscript{23} observed a higher prevalence of (EENV)\textsubscript{h} responders among Fulani and pointed out the association of this immune reactivity with one class II DQA-DQ\textsubscript{B} combination (serologic specificity DQw2) particularly common in the Fulani ethnic group. The fact that the Fulani showed higher humoral reactivity to all the \textit{P. falciparum} antigens tested\textsuperscript{3} (CSP, TRAP, MSA-1, Pf155/RESA, and Pf332), and at the same time displayed lower \textit{P. falciparum} parasite rates and incidence of clinical malaria,\textsuperscript{2} implies that the analysis of the possible protective role of these immune responses or of their possible association with particular HLA class II alleles should be stratified by ethnic group. Actually, if the data we present here had been analyzed disregarding the ethnic variable, a striking inverse association between all tested humoral responses and \textit{P. falciparum} parasite rate would have been obvious, solely as a result of the Fulani influence: in fact, we emphasize that such an association was not found when parasite rates and immune responses were examined within the same ethnic group.

The fact that the higher antibody response of the Fulani was observed for all antigens tested argues against an HLA class II-based explanation. A possible role of factors encoded at the same time displayed lower \textit{P. falciparum} parasite rates and incidence of clinical malaria,\textsuperscript{2} implies that the analysis of the possible protective role of these immune responses or of their possible association with particular HLA class II alleles should be stratified by ethnic group. Actually, if the data we present here had been analyzed disregarding the ethnic variable, a striking inverse association between all tested humoral responses and \textit{P. falciparum} parasite rate would have been obvious, solely as a result of the Fulani influence: in fact, we emphasize that such an association was not found when parasite rates and immune responses were examined within the same ethnic group.

The fact that the higher antibody response of the Fulani was observed for all antigens tested argues against an HLA class II-based explanation. A possible role of factors encoded by genes outside the HLA class II region in the regulation of humoral immune response against \textit{P. falciparum} antigens has been suggested by the study of twins by Sjoberg and others.\textsuperscript{24} Further possible mechanisms may involve interethic differences operating at levels such as B cell function, antigen processing,\textsuperscript{25} and expression of aspecific factors such as interleukin-4 (IL-4), which is an important modulator for the differentiation of precursor T helper cells to the Th2 or Th2-like cells that mediate humoral immunity and assist in antibody production.\textsuperscript{26,29} Troye-Blomberg and others\textsuperscript{30} reported that the production of IL-4 by activated human cells is associated with elevated levels of antibodies to malaria antigens. On this subject, it should be noted that Song and others\textsuperscript{31} recently showed that the human IL-4 promoter exists in multiple allelic forms that exhibit distinct transcriptional activities in IL-4-positive T cells. In other words, the possibility of the existence of genetically based quantitative rather than qualitative differences in immune responses to malaria antigens should be carefully considered. The hypothesis of a stronger general activation of the immune system in the Fulani is also suggested by the higher frequency in this ethnic group of the hyperreactive malarial splenomegaly syndrome.\textsuperscript{32,33}

The present data have direct relevance in malaria seroepidemiologic studies: overlooking human heterogeneities in the immune response and susceptibility to the infection, such as those reported in our study area, can be misleading in the evaluation of the protective role of antigen-specific immune responses to malaria and in serologically based estimations of malaria transmission. Moreover, the analysis of the immunogenetic mechanisms involved in the Fulani higher response to \textit{P. falciparum} is likely to improve our understanding of the host-parasite relationship and could influence the current approaches in vaccine research.

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