HUMORAL RESPONSE TO PLASMODIUM FALCIPARUM PF155/RING-INFECTED ERYTHROCYTE SURFACE ANTIGEN AND PF332 IN THREE SYMPATRIC ETHNIC GROUPS OF BURKINA FASO

DAVID MODIANO, ANTONELLA CHIUCCHIUINI, VINCENZO PETRARCA, BIENVENU SODIOMON SIRIMA, GAIA LUONI, HEDVIG PERLMANN, FULVIO ESPOSITO, AND MARIO COLUZZI

Dipartimento di Biologia Molecolare, Cellulare e Animale, Universita di Camerino, Camerino, Italy; Istituto di Parassitologia, World Health Organization Collaborating Centre for Malaria Epidemiology, Universita di Roma La Sapienza, Rome, Italy; Direzione Generale per la Cooperazione allo Sviluppo, Italian Ministry of Foreign Affairs, Rome, Italy; Centre National de Latte Contre le Paludisme, Ministere de la Sante, Ouagadougou, Burkina Faso; Department of Immunology, Stockholm University, Stockholm, Sweden

Abstract. The humoral immune response against synthetic peptides of two Plasmodium falciparum blood-stage antigens, PF155/ring-infected erythrocyte surface antigen (RESA) (EENV)6 and PF332 (SVTEEIAEEDK)2, 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 Caucasoid 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)6 and anti-(SVTEEIAEEDK)2 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 < 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 responses1–5 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 Caucasoid origin,1 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.2 The antigen contains two extensive blocks of tandemly repeated amino acid sequences, one in the C-terminus comprising five repeats of the octamer EENVHEDA 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 DDEH-VEEPTVA.5 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.9,15,16 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 Furadisme 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/Rimaiba 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 Rimaibe, 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 Rimaibe, 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 peptide increased with age in the three ethnic groups.

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 Rimaibe, 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 Rimaibe, who showed similar values. No intraethnic difference between sexes was recorded and no intragentic 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)$_5$ and anti-Pf332 (SVTEEIAEDK)$_2$ 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)$_5$ and anti-(SVTEEIAEDK)$_2$ 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 others23 observed a significant association between the presence of antibodies to the 3′ repeat region peptide (EENV)$_5$ 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-DQB combination (serologic specificity DQw2) particularly common in the Fulani ethnic group. The fact that the Fulani showed higher humoral reactivity to all P. falciparum antigens tested\textsuperscript{2} (CSP, TRAP, MSA-1, Pf155/RESA, and Pf332), and at the same time displayed lower 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 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 encoding of the host-parasite relationship and could influence the epidemiologic studies: overlooking human heterogeneities in malaria antigens should be carefully considered. The hypothesis of the existence of genetically based quantitative rather than qualitative differences in immune responses to malaria antigens should be carefully considered. The possibility of the existence of antigenic epitopes in the membrane of erythrocytes infected with early stages of Plasmodium falciparum, J Exp Med 159: 1686–1704.


Acknowledgments: We are grateful to the villagers of Barkoundouba and Barkoumbilen for kind participation throughout the investigation. We thank Dr. S. Ouali, Director of the Centre National de Lutte Contre le Paludisme (CNLP) of the Ministry of Health of Burkina Faso and Dr. Franco Pagnoni of the Italian Co-operation for support. We are deeply indebted to the personnel of the Immunoparasitology of the CNLP and particularly to Albert Yameogo for skilful technical assistance.

Financial support: The study was based at the CNLP of the Ministry of Health of Burkina Faso, supported by the Programma di Assistenza Tecnica della Direzione Generale per la Cooperazione allo Sviluppo of the Italian Ministry of Foreign Affairs. The study was partially supported by the World Health Organization, Division of Control of Tropical Diseases, and by the Fondazione Pasteur-Cenci Bolognetti of the University of Rome La Sapienza.

Authors’ addresses: David Modiano, Antonella Chiucchiuini, and Fulvio Esposito, Dipartimento di Biologia Molecolare, Cellulare e Animale, Universita di Camerino, Via F. Camerini 1, 62032, Camerino (MC), Italy. Vincenzo Petrarca, Gaia Luoni, and Mario Coluzzi, Istituto di Parasitologia, Universita La Sapienza, Piazzale Aldo Moro, 5, 00185 Rome Italy. Aldo Moro, 5, 00185 Rome Italy. Bienvenu Sodiomon Sirima, Centre National de Lutte Contre le Paludisme, Ministeres de la Sante, BP 2208, Ouagadougou, Burkina Faso. Hedwig Perlmann, Department of Immunology, Stockholm University, S-106 91 Stockholm, Sweden.

Reprint requests: David Modiano, Istituto di Parassitologia, Universita La Sapienza, Piazzale Aldo Moro, 5, 00185 Rome Italy.

REFERENCES


