Am. J. Trop. Med. Hyg., 74(6), 2006, pp. 979–985

ANTIBODY RESPONSES TO SEVERAL MALARIA PRE-ERYTHROCYTIC ANTIGENS AS A MARKER OF MALARIA EXPOSURE AMONG TRAVELERS

EVE ORLANDI-PRADINES, KRISTELL PENHOAT, CLAUDE DURAND, CHRISTOPHE PONS, CHRISTIAN BAY, BRUNO PRADINES, THIERRY FUSAI, RICHARD JOSSE, PHILIPPE DUBROUS, JEAN-BAPTISTE MEYNARD, JEAN-PAUL DURAND, RENE MIGLIANI, JEAN-PAUL BOUTIN, PIERRE DRUILHE, AND CHRISTOPHE ROGIER*

Unité de Recherche en Biologie et en Épidémiologie Parasitaires, Département d'Épidémiologie et de Santé Publique Sud, et Unité de Virologie, Institut de Médecine Tropicale du Service de Santé des Armées, Marseille, France; Institut Fédératif de la Recherche 48, Marseille, France; Service de Santé du Premier Régiment de Chasseurs Parachutistes, Pamiers, France; Département d'Épidémiologie et Laboratoire de Biologie Médicale de l'Hôpital d'Instruction des Armées Robert Picqué, Bordeaux, France; Laboratoire de Parasitologie Bio-Médicale, Institut Pasteur, Paris, France

Abstract. Protective devices against vectors are used by travelers in malaria-endemic areas but their efficacy for protection against mosquitoes has rarely been evaluated. The level of exposure to malaria transmission of 205 soldiers deployed in Africa and the efficacy of their anti-vector prophylaxis was evaluated by comparison of their IgM and IgG responses against five pre-erythrocytic *Plasmodium falciparum* antigens (circumsporozoite protein, sporozoite threonine- and asparagine-rich protein, sporozoite- and liver-stage antigen, liver stage antigen 1, and SR11.1) before and at the end of their deployment, and three months after returning to France for 106 of these soldiers. The immune responses increased significantly during the mission in 35% (95% confidence interval = 28–42%) of the individuals. The permanent use of insecticide-treated bed nets and long-sleeve battle dress at night were associated with protective efficacy. The analysis of these antibody responses was sensitive enough to evaluate exposure to malaria transmission and the efficacy of anti-vector devices in travelers using antimalarial chemoprophylaxis.

INTRODUCTION

Imported malaria is a growing problem in non-endemic areas throughout the world. Annually, up to 10,000 cases of malaria in Europe[1] and 30,000 cases in all industrialized countries are imported with an average case fatality rate ranging from 1% to 4%.[2,3] Chemoprophylaxis and anti-vector devices such as impregnated bed nets, repellents, and long-sleeve clothes have been proposed as protective measures for non-immune people traveling in malaria-endemic areas.[4] The level and frequency of *Plasmodium falciparum* and *P. vivax* drug resistance in numerous areas,[5] poor tolerance to some antimalarial drugs,[6] and difficulties in ensuring good compliance with prophylactic regimens[7] emphasize the importance of anti-vector devices. However, their use is often considered restrictive and their efficacy for protecting travelers has rarely been evaluated. Entomologic methods are not designed for estimating the individual level of exposure to malaria transmission and their use is not practical for evaluation of the efficacy of anti-vector devices among travelers.

In contrast, serologic methods may provide an indirect means of estimating individual exposure to malaria transmission. Antibody response to sporozoites has been studied extensively in immune populations[8–10] and in non-immune travelers using chemoprophylaxis.[11–13] Most frequently, this analysis is performed with enzyme-linked immunosorbent assay (ELISA) techniques based on synthetic peptides or recombinant proteins including the repetitive NANP and NVDP domains of the circumsporozoite protein (CSP).[14] It has frequently been used as a proxy for the evaluation of malaria transmission.[15–17]

The association between antibody responses to other pre-erythrocytic and erythrocytic stage antigens and level of malaria transmission has been studied in immune populations.[18–21] However, the antibody response to these antigens in non-immune travelers briefly exposed to malaria has not been extensively studied. Our objective was to estimate the level of exposure to malaria transmission of French soldiers deployed to tropical Africa by analyzing their antibody responses against several pre-erythrocytic stages antigens. Secondary objectives were to analyze the kinetics of these antibodies and to evaluate the efficacy of individual anti-vector devices. These antigens include CSP, which is actively expressed only in the sporozoite stage and is generally used as a reference for estimation of immunologic exposure to malaria transmission.[15] The pre-erythrocytic antigens tested also included liver stage antigen 1 (LSA1), which is expressed only in the hepatic stage, sporozoite threonine- and asparagine-rich protein (STARP), and sporozoite- and liver-stage antigen (SALSA), which is expressed both at the sporozoite and hepatic stages.[22] A high prevalence of antibody responses to these pre-erythrocytic antigens has been found in individuals living in malaria-endemic areas.[23–25] The antibody response directed against SR11.1 antigen, a novel antigen that is expressed at sporozoite and liver stages and corresponds to a unique subregion of the megaprotein *Pf* 11.1 (Brahimi K and others, unpublished data and Perlaza BL and others, unpublished data), was also studied. Our findings may be useful in understanding the first stages of the immune responses to primary malaria infection and developing vaccines against malaria.

MATERIALS AND METHODS

Persons and blood samples. The antibody response against *P. falciparum* antigens was studied in 205 French soldiers on a five-month mission in Gabon from June to September 2002 (dry season), and in Côte d'Ivoire from October to November 2002 (rainy season). Gabon and Côte d'Ivoire are areas endemic for *P. falciparum*. Blood samples were collected in May 2002 (T0), December 2002 (one week after returning to

* Address correspondence to Christophe Rogier, Unité de Recherche en Biologie et Épidémiologie Parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Boulevard Charles Livon, Parc le Pharo, BP 46, 13998 Marseille Armées, France and Institut Fédératif de la Recherche 48, 13005 Marseille, France. E-mail: christophe.rogier@wanadoo.fr

France, T1) and March 2003 (three months later in France, T2). Sera were separated and stored at –20°C. A questionnaire was completed by all subjects at T1 to determine age, time spent in malaria-endemic areas before the mission, history of acute clinical malaria attacks before and during the mission, and compliance with mandatory anti-malaria prophylaxis. The latter included chemoprophylaxis (350 mg of mefloquine per week in Gabon and 100 mg of doxycycline per day in Côte d'Ivoire and during the four-week period after returning to France) and use of permethrin-impregnated bednets, repellents, and long-sleeve battle dress at night. The Army provided prophylactic drugs, anti-vector equipment, and repellents. In Gabon, the soldiers were involved in training activities and usually slept in permanent urban structures, most of which were air-conditioned and mosquito-proof. In Côte d'Ivoire, they took part in fighting and peacekeeping operations. During that period, they lived in rough rural conditions and often slept in damaged houses or bivouacs. Blood samples obtained twice at a 3–5-month interval from 46 individuals living in Marseille, France, i.e., without exposure to malaria transmission, were used as controls. All participants provided informed consent before participating in the study. The study protocol was reviewed and approved by the Marseille-2 ethical committee.

Peptides. A 24-mer peptide containing six copies of tandem repeating domains $(NANP)_4(NVDP)_2$ of CSP, a 43-mer peptide containing two copies of the central tandem repeating domain of LSA1 [LAKEKLQ(EQQSDLEQERLAKEKEKLQ)$_2$],[23] a 20-mer peptide containing two copies of tandem repeating domain $(STNNNTKTI)_2$ of STARP,[25] a 34-mer peptide containing the non-repeat region NGKDDVKEEKKTNEKKDDGKTDKVQEKVLEKSPK of SALSA,[24] and an 18-mer consensus SR11.1-derived peptide (EEVVVVLIEEVIPEELVL) containing the sequence found most often among the repeats of the megaprotein Pf 11.1 (Brahimi K and others, unpublished data and Perlaza BL and others, unpublished data) were synthesized and purified (> 90%) with Genosys (Sigma-Genosys, Cambridge, United Kingdom) with an added N-terminal biotin. All peptides were shipped lyophilized. They were resuspended in dimethyl sulfoxide (Sigma Chemical Co., St Louis, MO, USA) and stored in aliquots at –20°C.

Enzyme-linked immunosorbent assay. IgG and IgM antibodies to CSP, LSA1, STARP, SALSA, and SR11.1 were tested with an ELISA. Flat-bottom microtiter plates (Nunc, Roskilde, Denmark) were coated with 100 μL/well of streptavidin (5 μg/mL) (Roche, Basel, Switzerland) overnight at 37°C. The plates were then blocked for one hour at 40°C with 200 μL of blocking buffer, pH 6.6 (phosphate-buffered saline [PBS], pH 7.4, 0.1% Tween 20 [Sigma Chemical Co.], 3% skim milk [Becton Dickinson Bioscience, San Jose, CA]) and washed three times with PBS, pH 7.2, 0.1% Tween 20 (PBS-Tween). Biotinylated synthetic peptides were added to the plates (100 μL of individual peptide [2.5 μM in PBS-Tween]) and incubated for one hour at 40°C. The plates were then washed three times with PBS-Tween. Sera were diluted 1:200 in blocking buffer. For the detection of IgM antibodies, sera were first diluted 1:25 in PBS, then mixed with rheumatoid factor absorbent (Biomérieux SA, Craponne, France) to a 1:50 serum dilution and incubated for one hour at room temperature. The mixture was centrifuged for 10 minutes at 9,000 × *g* and the supernatant was mixed with blocking buffer to give a final serum dilution of 1:200. Diluted sera were incubated (100 μL/well) for one hour at 40°C and plates were then washed three times with PBS-Tween. For detecting human IgG and IgM, plates were incubated for one hour at 40°C with 100 μL of peroxidase-conjugated goat $F(ab')_2$ fragment to human IgG Fc (Cappel; ICN Biomedicals, Aurora, OH) diluted 1:60,000 in blocking buffer or with peroxidase-conjugated goat $F(ab')_2$ fragment to human IgM (Cappel, ICN Biomedicals, OH, USA) diluted 1:80,000 in the blocking buffer, respectively. Plates were then washed three times with PBS-Tween and incubated for 15 minutes at room temperature with 80 μL of tetramethylbenzidine substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The reaction was stopped by addition of 50 μL of 1M H_2SO_4. The plates were read at 450 nm with an ELISA reader (Versa Max® turnable multiplate reader; Molecular Devices, Wokingham, United Kingdom).

Each serum was tested in duplicate on each peptide and in control wells without peptide. The serum was tested again when the absorbance in the control well was greater than 0.30 or when one duplicate had a signal ≥ 50% of the other. All serum samples from one individual were tested together on the same plate. A pool of sera of European adults who had never been exposed to malaria and a pool of sera of African adults exposed to malaria transmission were used in each assay as negative and positive controls, respectively. Inter-assay variations in absorbance values of the negative and positive controls ranged within 25% of their mean values.

The levels of IgG and IgM antibodies to each antigen were expressed as adjusted optical densities (ODs). The latter were calculated for each peptide and each serum as the ratio of the mean OD value with peptide divided by the OD value of the control wells, i.e., without peptide. The adjusted OD for IgG and IgM to each antigen for 48 sera from European adults who had never been exposed to malaria were normalized using the Box-Cox transformation, and their normalized mean and SD were estimated. The lower limit of positivity for IgG and IgM to each antigen was taken as the reverse transformation of the mean + 2.3263 SD estimated on Box-Cox transformed data. Therefore, values higher than the cut-offs should be observed in less than 1% of the sera of non-exposed individuals. The thresholds of positivity were 2.15, 1.54, 1.47, 2.32, and 1.58, respectively, for IgG antibodies to SR11.1, CSP, LSA1, SALSA, and STARP and 2.31, 1.53, 1.32, 1.81, and 2.44, respectively, for IgM antibodies to these five antigens.

The variations in IgG and IgM responses to each antigen in absence of exposure to malaria transmission were assessed by the ratio of adjusted ODs on 45 pairs of sera collected at 3–5-month intervals from unexposed European adults living permanently in Marseille, France, i.e., in non-endemic area. The values of these ratios were normalized using the Box-Cox transformation, and their normalized mean and SD were estimated. The lower limit of significant increase in IgG and IgM responses to each antigen was taken as the reverse transformation of the mean ratio of the corrected OD + 3.0902 SD estimated on Box-Cox transformed data. Values greater than these cut-offs should be observed in less than 0.1% of the pairs of sera collected at 3–5-month intervals in unexposed individuals. The thresholds defining significant increases in antibody responses to SR11.1, CSP, LSA1, SALSA, and STARP were 1.63, 1.57, 1.50, 1.43, and 1.26, for IgG antibody

ratios and 1.34, 1.19, 1.39, 1.40 and 1.37, respectively, for IgM antibody ratios, respectively.

Statistical analysis. Statistical analysis was performed with Stata version 7.0 (Stata Corporation, College Station, TX). The Wilcoxon signed rank test and Mann-Whitney rank sum test were used to compare variables between groups for paired and unpaired data, respectively. The Fisher exact test was used to compare proportions. The McNemar exact test was used to compare incidence rates of significant increase in immune response between tested antigens. Pairwise correlations between the IgG and IgM antibody responses at T1 were tested using the Bonferroni correction for the estimation of the *P* value to take into account the multiplicity of the tests. Multivariate analysis was performed by fitting logistic regression models with a significant increase in the IgG or IgM response to at least one antigen as the dependent variable. Differences were considered statistically significant when $P < 0.05$.

RESULTS

The mean age of the 205 male soldiers in the French Army enrolled in the study was 25 years (25–75% percentile = 23–27 years, range = 18–45 years). Among the 205 volunteers, 123 (60%) had previously traveled to malaria-endemic areas. During the mission, compliance with chemoprophylaxis, use of repellents, and use of long-sleeve clothes was reported by 160 (78%), 42 (20.5%), and 66 (32%) soldiers, respectively. Sleeping under insecticide-impregnated bed nets was reported by 145 (71%) soldiers. Eight soldiers reported a *P. falciparum* clinical malaria attack before the mission, four during the mission, and three after the mission. Six individuals experienced a *P. ovale* clinical malaria attack after their return to France.

The number of volunteers reported compliance during the three cross-sectional surveys was 205 at T0, 205 at T1, and 106 at T2. The IgG and IgM antibody responses to SR11.1, CSP, LSA1, SALSA, and STARP of the 106 individuals sampled in the three surveys are shown in Figure 1. Although the IgG and IgM adjusted ODs (Figure 1A) generally increased from T0 to T1 and then decreased from T1 to T2, only the IgG and IgM responses against LSA1 increased significantly ($P < 0.001$) from T0 to T1, and decreased significantly from T1 to T2 (IgG; $P = 0.03$ and IgM; $P < 0.001$). Increased IgG and IgM responses to LSA1 were observed among individuals who never traveled to malaria-endemic areas (mean IgG adjusted OD = 1.05 at T0 versus 2.07 at T1, n = 82, $P < 0.001$ by Wilcoxon signed rank test and mean IgM adjusted OD = 1.05 at T0 versus 1.66 at T1, n = 82; $P < 0.001$) and among individuals who stayed in malaria-endemic areas (mean IgG adjusted OD = 1.09 at T0 versus 2.69 at T1, n = 123; $P < 0.001$ and mean IgM adjusted OD = 1.07 at T0 versus 1.65 at T1, n = 122; $P < 0.001$).

The prevalence rates of positivity for IgG to SR11.1, CSP, LSA1, SALSA, and STARP at T0 were 2.9% (95% confidence interval [CI] = 0.6–8.13%), 4.8% (95% CI = 1.6–10.8%), 6.7% (95% CI = 2.7–13.%), 6.7% (95% CI = 2.7–13.3%), and 3.8% (95% CI = 1.0–9.5%), respectively (Figure 1B). The prevalence rates of positivity for IgM at T0 to the same antigens were less than 2% except for IgM to SALSA

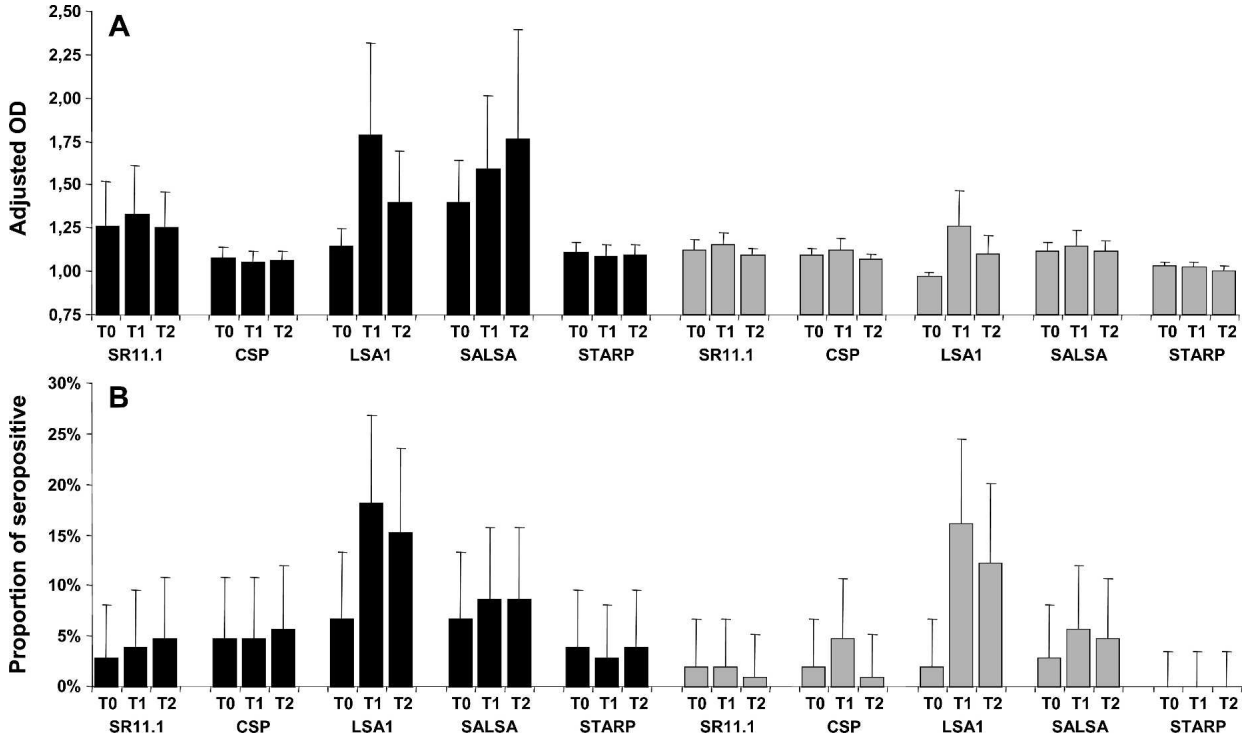

FIGURE 1. Antibody responses to SR11.1, circumsporozoite protein (CSP), liver stage antigen 1 (LSA1), sporozoite- and liver stage antigen (SALSA), and sporozoite threonine- and asparagine-rich protein (STARP) in soldiers before (T0), one week after (T1), and three months after (T2) a five-month deployment in Gabon (June–September 2002) and Côte d'Ivoire (October–November 2002). IgG (black bars, n = 105) and IgM (gray bars, n = 106) responses are shown. **A**, Adjusted optical density (OD) - OD with peptide/OD without peptide. **B**, Proportion of seropositive persons. Error bars show the upper limits of the 95% confidence intervals.

(2.8%, 95% CI = 0.6–8%). Overall, the prevalence rate of positivity for IgG or IgM antibody to any of the antigens at T0 was 32% (95% CI = 26–39%). At T1, there was a significant correlation between the adjusted OD of the IgG responses to LSA1 and SR11.1 (r = 0.215, $P = 0.0197$) or CSP (r = 0.283, $P = 0.0004$), between the adjusted OD of the IgM responses to LSA1 and SR11.1 (r = 0.474, $P = 0.0001$), CSP (r = 0.291, $P = 0.0002$), or SALSA (r = 0.332, $P = 0.0001$), and between the adjusted OD of the IgM responses to SALSA and SR11.1 (r = 0.259, $P = 0.0018$) or STARP (r = 0.224, $P = 0.0127$).

The incidence rates of significant increase in antibody responses to SR11.1, CSP, LSA1, SALSA, and STARP between T0 and T1 are shown in Figure 2. The IgG and the IgM responses against at least one antigen increased significantly in 23.4% (95% CI = 17.8–29.8%) and 25.4% (95% CI = 19.6–31.9%), respectively. Taken together, 34.6% (95% CI = 28.1–41.6%) of the individuals had a significant increase in IgG or IgM to at least one antigen between T0 and T1. The number of individuals with significantly increased antibody responses to one, two, three, four, and five antigens were 29, 10, 5, 3, and 1 for IgG (n = 205) and 27, 14, 8, 2, and 0 for IgM (n = 204), respectively. Among the 71 individuals with a significant increase in IgG or IgM responses to at least one antigen between T0 and T1, 57 (80%), 24 (34%), 21 (30%), 19 (27%), and 16 (22%) showed an increased IgG or IgM antibody response to LSA1, SR11.1, SALSA, CSP, and STARP, respectively. Moreover, 89% (63 of 71) and 94% (67 of 71) showed a significant increase in IgG or IgM responses to LSA1 and STARP, and LSA1, STARP, and CSP, respectively. The incidence rate of increased IgG and IgM antibody responses to LSA1 were significantly higher than those to any other antigen ($P < 0.0001$), and 27.8% (95% CI = 21.8–34.5%) of the individuals had a significant increase in IgG or IgM antibodies to LSA1. The incidence rate of increased IgM antibody response to STARP was significantly lower than the incidence rates of increased IgM antibody responses to CSP and SR11.1 ($P = 0.03$ and $P = 0.01$). A significant increase in IgG or IgM antibodies to at least one antigen was observed between T0 and T1 in three of four individuals who experienced a clinical malaria attack during the mission.

The incidence rate of increased IgG or IgM antibody responses to at least one antigen is shown in Table 1 according to age, previous stay in malaria-endemic areas, serologic status before the mission, compliance with chemoprophylaxis, and use of anti-vector devices. Multivariate logistic regression analysis showed good compliance for the use of long-sleeve clothes at night (odds ratio [OR] = 0.34, 95% CI = 0.12–0.93, $P = 0.036$) and use of an insecticide-impregnated bed net during the mission in Côte d'Ivoire (OR = 0.40, 95% CI = 0.21–0.75, $P = 0.004$) that were significantly and independently associated with a lower incidence of increased antibody responses to *P. falciparum* antigens during the mission. The adjusted protective efficacy of regular use of long-sleeve clothes and impregnated bed nets were 66% and 60%, respectively.

The incidence rate of increased IgG or IgM antibody response to LSA1 did not differ significantly according to age, previous stay in malaria-endemic areas, serologic status before the mission, compliance with chemoprophylaxis, or use of repellents. Multivariate logistic regression analysis showed that it differed significantly with the use of long-sleeve clothes (poor compliance as reference = 45%, some omissions = 28%, OR = 0.47, 95% CI = 0.18–1.22, $P = 0.123$; good compliance = 21%, OR = 0.32, 95% CI = 0.11–0.92, $P = 0.035$) and use of insecticide-impregnated bed nets during the mission in Côte d'Ivoire (not regular use as reference = 42%, regular use = 22%, OR = 0.39, 95% CI = 0.21–0.77, $P = 0.006$).

Previous stay in malaria-endemic areas or seropositivity to at least one antigen before the mission had no confounding or interaction effect on the incidence rate of increased IgG or IgM antibody responses to LSA1 or to at least one antigen. Moreover, the incidence of clinical malaria attacks (*P. falciparum* or *P. ovale*) was significantly associated with an increased IgG or IgM antibody response to at least one antigen between T0 and T1 (OR = 1.45, 95% CI = 1.08–1.94, $P = 0.014$).

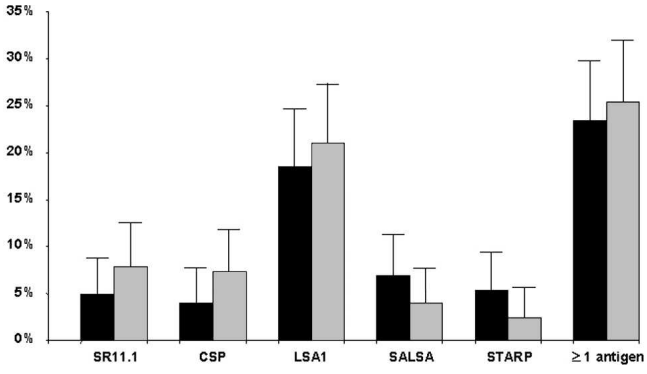

FIGURE 2. Incidence of an increase in antibody responses to SR11.1, circumsporozoite protein (CSP), liver stage antigen 1 (LSA1), sporozoite- and liver stage antigen (SALSA), and sporozoite threonine- and asparagine-rich protein (STARP) in soldiers between before (T0) and after (T1) a five-month deployment in Gabon (June–September 2002) and Côte d'Ivoire (October–November 2002). IgG (black bars, n = 205) and IgM (gray bars, n = 204) responses are shown. Error bars show the upper limits of the 95% confidence intervals.

DISCUSSION

Although exposure to malaria transmission in the study population was short and probably low because of the use of anti-vector devices, approximately 35% of the individuals reacted to at least one of the antigens tested. Only 2% of the soldiers had experienced a clinical malaria attack during the mission. One can speculate that the individuals with a significant increase in IgG or IgM antibody response to at least one antigen between T0 and T1 were recently infected by *P. falciparum* and most were protected from clinical malaria by effective chemoprophylaxis. The peptides tested were derived from LSA1,[23,26] CSP,[14] STARP,[25] and SALSA,[24] which have been shown to be antigenic in humans living in malaria-endemic areas. IgG antibody to $(NANP)_n$ has been extensively used as a marker of exposure to malaria transmission in immune populations[8–10] but rarely in non-immune short-term travelers using chemoprophylaxis.[11–13] In the present study, 6.8% of the soldiers showed a significant increase in IgG response to CSP after returning from malaria-endemic areas. This figure is similar to the previously estimated positivity

TABLE 1

Incidence rate of increased IgG or IgM antibody responses to at least one of five antigens (SR11.1, CSP, LSA1, SALSA or STARP) in soldiers during a five-month deployment to Gabon (June–September 2002) and Côte d'Ivoire (October–November 2002) according to age, previous stay in malaria-endemic areas, serologic status before travel, compliance with chemoprophylaxis, and the use of antivectorial devices*

	IgG or IgM response to at least one antigen				Crude	95%		Adjusted	95%	
	Negative	Positive	% Positive	Total	OR	CI	*P*	OR	CI	*P*
No. of subjects	134	71		205						
Age, years										
< 25	56	33	37.1	89	1					
≥ 25	78	38	32.8	116	0.83	0.46 1.48	0.520			
Previous stay in malaria-endemic areas										
No	50	32	39.0	82	1					
Yes	84	39	31.7	123	0.73	0.40 1.30	0.281			
Positive for IgG or IgM to any tested antigens before the mission										
No	90	49	35.3	139	1					
Yes	44	22	33.3	66	1.03	0.57 1.85	0.930			
Chemoprophylaxis										
Poor compliance	4	3	42.9	7	1					
Some omissions	19	19	50.0	38	1.33	0.26 6.78	0.729			
Good compliance	111	49	30.6	160	0.59	0.13 2.73	0.498			
Use of repellents										
Poor compliance	57	34	37.4	91	1					
Some omissions	51	21	29.2	72	0.69	0.36 1.34	0.273			
Good compliance	26	16	38.1	42	1.03	0.49 2.19	0.935			
Use of long-sleeved clothes										
Poor compliance	10	12	54.5	22	1			1		
Some omissions	77	40	34.2	117	0.43	0.17 1.09	0.075	0.43	0.17 1.13	0.086
Good compliance	47	19	28.8	66	0.34	0.12 0.91	0.032	0.34	0.12 0.93	0.036
Use of bed nets in Gabon										
Not regular	37	23	38.3	60	1			1		
Regular	97	48	33.1	145	0.80	0.43 1.49	0.474	0.91	0.47 1.77	0.780
Use of bed nets in Côte d'Ivoire										
Not regular	30	30	50.0	60	1			1		
Regular	104	41	28.3	145	0.39	0.21 0.73	0.003	0.40	0.21 0.75	0.004

* CSP = circumsporozoite protein; LSA1 = liver stage antigen 1; SALSA = sporozoite- and liver-stage antigen; STARP = sporozoite threonine- and asparagine-rich protein; OR = odds ratio; CI = confidence interval.

rate of 4.9% in 2,131 travelers returning from malaria-endemic areas[27] or in 262 travelers returning from Kenya.[13] The simultaneous use of several antigens, compared with using only one antigen, i.e., CSP, resulted in higher serologic estimates of exposure to malaria transmission. Our data suggest that two or three antigens are necessary to detect approximately 90% and 95% of the antibody responses to at least one of the five antigens, respectively. Even if our study was not designed to compare these antigens for the assessment of malaria exposure, antibodies to LSA1, rather than antibodies to CSP, result in a more accurate assessment and more than one antigen should be used in testing non-immune short-term travelers.

The CSP, SR11.1, STARP, and SALSA peptides gave similar estimates, whereas the LSA1 peptide showed a much higher incidence rate of antibody response. The synthetic peptide corresponding to the LSA1 antigen was the longest (43 amino acids) and could represent a better conformation of antigen or could correspond to several epitopes. Alternatively, epidemiologic studies have shown a consistently high antigenicity of LSA1 repeats.[23,25] Most likely, the use of the native proteins or the full-length recombinant proteins would increase the sensitivity of the method for detecting *P. falciparum* infections. There was a poor correlation between the T1 antibody responses to the different antigens. Thus, increasing the number of antigens to may provide a better estimation of the level of malaria transmission.

The use of paired sera from each individual, i.e. before and after exposure to malaria transmission, allows one to consider each subject as its own control and markedly improves the sensitivity of detection of an antibody response. We used paired sera from unexposed individuals to estimate cut-off values defining the significance level of such a response. The high level of the estimated cut-off that should be reached by less than 0.1% of unexposed individuals and the use of paired sera from each individual contribute to a highly specific criteria for a positive antibody response. This cut-off may not be considered pertinent for secondary antibody responses in individuals who were seropositive before the exposure. However, the incidence rate of significantly increased IgG or IgM antibody responses did not differ between individuals regardless of their serologic status before exposure to malaria transmission or their history of time in malaria-endemic areas, which suggests that the cut-offs were appropriate.

It is important to conduct blood sampling early after the end of exposure to malaria transmission. Antibody titers to LSA1 decreased significantly between the time of arrival in France and two months later. The decrease in antibodies to the other antigens was not significant. Conversely, there was a non-significant increase in IgG antibodies to SALSA during the same period.

Collecting behavior data with a retrospective questionnaire may result in omissions or memory biases. However, it is likely that the omissions or the flaws in remembering would bias the estimates of the effect of the mandatory prophylactic measures to zero. Our study has shown that anti-vector de-

vices are protective against infection in travelers. Permanent night use of impregnated bed nets in Côte d'Ivoire and long-sleeve clothes had protective efficacies of 60% and 66%, respectively. The absence of a protective effect of the use of bed nets in Gabon may be explained by a much lower exposure to mosquitoes in the urban environment and in mosquitoes-proof houses in Libreville. Serologic estimation of exposure to malaria transmission used in this study was sensitive enough to detect the protective effect of anti-vector devices, such as long-sleeve clothes, even among short-term travelers.

Received November 17, 2005. Accepted for publication January 18, 2006.

Acknowledgments: We thank all volunteers and the medical team of Régiment de Chasseur Parachutiste (Pamiers, France) for participating in the study. We also thank F. Remoue (Institut de Recherche et de Développement, Dakar, Senegal) and A. Buguet for critically reading the manuscript and C. Dane (Délégation Générale pour l'Armement, Paris, France) for support and advice.

Financial support: This study was supported by Délégation Générale pour l'Armement (grant 02CO011, no. 010808).

Author' addresses: Eve Orlandi-Pradines, Kristell Penhoat, Claude Durand, Bruno Pradines, Thierry Fusai, and Christophe Rogier, Unité de Recherche en Biologie et Épidémiologie Parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Boulevard Charles Livon, Parc le Pharo, BP 46, 13998 Marseille Armées, France and Institut Fédératif de la Recherche 48, 13005 Marseille, France, Telephone: 33-4-91-15-01-50, Fax: 33-4-91-15-01-64. E-mails: sururu@caramail.com, bruno.pradines@free.fr, thierry.fusai@free.fr, and christophe.rogier@wanadoo.fr. Christophe Pons and Christian Bay, Service de Santé du Premier Régiment de Chasseurs Parachutistes, Quartier Capitaine Beaumont, 09105 Pamiers, France, Telephone: 33-5-34-01-99-99, Fax: 33-5-34-01-99-48. Richard Josse, Département d'Épidémiologie de l'Hôpital d'Instruction des Armées Robert Picqué, Bordeaux, France, Telephone: 33-5-56-84-70-42, Fax: 33-5-56-84-72-90. Philippe Dubrous, Laboratoire de Biologie Médicale de l'Hôpital d'Instruction des Armées Robert Picqué, Bordeaux, France, Telephone: 33-5-56-84-74-27, Fax: 33-5-56-84-72-90. Jean-Baptiste Meynard, Rene Migliani and Jean-Paul Boutin, Département d'Épidémiologie et de Santé Publique Sud, Institut de Médecine Tropicale du Service de Santé des Armées, Boulevard Charles Livon, Parc le Pharo, BP 46, 13998 Marseille Armées, France and Institut Fédératif de la Recherche 48, 13005 Marseille, France, Telephone: 33-4-91-15-25, Fax: 33-4-91-15-01-84, E-mail: imtssa.desp@wanadoo.fr. Jean-Paul Durand, Unité de Virologie, Institut de Médecine Tropicale du Service de Santé des Armées, Boulevard Charles Livon, Parc le Pharo, BP 46, 13998 Marseille Armées, France. Pierre Druilhe, Laboratoire de Parasitologie Bio-Médicale, Institut Pasteur, 28 Rue du Dr. Roux, 75015 Paris, France, Telephone: 33-1-45-68-85-78, Fax: 33-1-45-68-86-40, E-mail: druilhe@pasteur.fr.

REFERENCES

1. World Health Organization, 2004. *International Travel and Health: Vaccination Requirements and Health Advice.* Available from www.who.int/ith/
2. Muentener P, Schlagenhauf P, Steffen R, 1999. Imported malaria (1985–95): trends and perspectives. *Bull World Health Organ 77:* 560–566.
3. Kain KC, Keystone JS, 1998. Malaria in travelers. Epidemiology, disease, and prevention. *Infect Dis Clin North Am 12:* 267–284.
4. Chen LH, Keystone JS, 2005. New strategies for the prevention of malaria in travelers. *Infect Dis Clin North Am 19:* 185–210.
5. Wernsdorfer WH, 1991. The development and spread of drug-resistant malaria. *Parasitol Today 7:* 297–303.
6. Schlagenhauf P, Tschopp A, Johnson R, Nothdurft HD, Beck B, Schwartz E, Herold M, Krebs B, Veit O, Allwinn R, Steffen R, 2003. Tolerability of malaria chemoprophylaxis in non-immune travellers to sub-Saharan Africa: multicentre, randomised, double blind, four arm study. *BMJ 327:* 1078.
7. Lobel HO, Phillips-Howard PA, Brandling-Bennett AD, Steffen R, Campbell CC, Huong AY, Were JB, Moser R, 1990. Malaria incidence and prevention among European and north American travellers to Kenya. *Bull World Health Organ 68:* 209–215.
8. Archibald CP, Mak JW, Mathias RG, Selvajothi S, 1990. Antibodies to *Plasmodium falciparum* in an indigenous population from a malaria endemic area of Malaysia. *Acta Trop 48:* 149–157.
9. 1992. The level of anti-sporozoite antibodies in a highly endemic malaria area and its relationship with exposure to mosquitoes. Kilombero Malaria Project. *Trans R Soc Trop Med Hyg 86:* 499–504.
10. Romi R, Sabatinelli G, Majori G, Ralamboranto L, Raveloarifera F, Ranaivoharimina H, 1994. *Plasmodium falciparum* circumsporozoite antibody prevalence in Madagascar: a longitudinal study in three different epidemiologic areas. *Am J Trop Med Hyg 51:* 856–863.
11. Cobelens FG, Verhave JP, Leentvaar-Kuijpers A, Kager PA, 1998. Testing for anti-circumsporozoite and anti-blood-stage antibodies for epidemiologic assessment of *Plasmodium falciparum* infection in travelers. *Am J Trop Med Hyg 58:* 75–80.
12. Bwire R, Slootman EJ, Verhave JP, Bruins J, Docters van Leeuwen WM, 1998. Malaria anticircumsporozoite antibodies in Dutch soldiers returning from sub-Saharan Africa. *Trop Med Int Health 3:* 66–69.
13. Nothdurft HD, Jelinek T, Blum LA, von Sonnenburg F, Loscher T, 1999. Seroconversion to circumsporozoite antigen of *Plasmodium falciparum* demonstrates a high risk of malaria transmission in travelers to east Africa. *Clin Infect Dis 28:* 641–642.
14. Zavala F, Tam JP, Masuda A, 1986. Synthetic peptides as antigens for the detection of humoral immunity to *Plasmodium falciparum* sporozoites. *J Immunol Methods 93:* 55–61.
15. Druilhe P, Pradier O, Marc JP, Miltgen F, Mazier D, Parent G, 1986. Levels of antibodies to *Plasmodium falciparum* sporozoite surface antigens reflect malaria transmission rates and are persistent in the absence of reinfection. *Infect Immun 53:* 393–397.
16. Jelinek T, Nothdurft HD, Loscher T, 1995. Evaluation of circumsporozoite antibody testing as a sero-epidemiological tool for the detection of *Plasmodium falciparum* infection in non-immune travelers. *Trop Med Parasitol 46:* 154–157.
17. Webster HK, Gingrich JB, Wongsrichanalai C, Tulyayon S, Suvarnamani A, Sookto P, Permpanich B, 1992. Circumsporozoite antibody as a serologic marker of *Plasmodium falciparum* transmission. *Am J Trop Med Hyg 47:* 489–497.
18. 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.
19. Migot-Nabias F, Deloron P, Ringwald P, Dubois B, Mayombo J, Minh TN, Fievet N, Millet P, Luty A, 2000. Immune response to *Plasmodium falciparum* liver stage antigen-1: geographical variations within Central Africa and their relationship with protection from clinical malaria. *Trans R Soc Trop Med Hyg 94:* 557–562.
20. Meraldi V, Nebie I, Tiono AB, Diallo D, Sanogo E, Theisen M, Druilhe P, Corradin G, Moret R, Sirima BS, 2004. Natural antibody response to *Plasmodium falciparum* Exp-1, MSP-3 and GLURP long synthetic peptides and association with protection. *Parasite Immunol 26:* 265–272.
21. Drakeley CJ, Corran PH, Coleman PG, Tongren JE, McDonald SL, Carneiro I, Malima R, Lusingu J, Manjurano A, Nkya WM, Lemnge MM, Cox J, Reyburn H, Riley EM, 2005. Estimating medium- and long-term trends in malaria transmission by using serological markers of malaria exposure. *Proc Natl Acad Sci U S A 102:* 5108–5113.
22. Druilhe PRL, Fidock D, 1998. Immunity to liver stages. Sherman IW, ed. *Malaria: Parasite Biology, Pathogenesis and Protection*. Washington, DC: American Society for Microbiology Press, 513–540.
23. Fidock DA, Gras-Masse H, Lepers JP, Brahimi K, Benmohamed

L, Mellouk S, Guerin-Marchand C, Londono A, Raharimalala L, Meis JF, 1994. *Plasmodium falciparum* liver stage antigen-1 is well conserved and contains potent B and T cell determinants. *J Immunol 153:* 190–204.

24. Bottius E, BenMohamed L, Brahimi K, Gras H, Lepers JP, Raharimalala L, Aikawa M, Meis J, Slierendregt B, Tartar A, Thomas A, Druilhe P, 1996. A novel *Plasmodium falciparum* sporozoite and liver stage antigen (SALSA) defines major B, T helper, and CTL epitopes. *J Immunol 156:* 2874–2884.
25. Pasquetto V, Fidock DA, Gras H, Badell E, Eling W, Ballou WR, Belghiti J, Tartar A, Druilhe P, 1997. *Plasmodium falciparum* sporozoite invasion is inhibited by naturally acquired or experimentally induced polyclonal antibodies to the STARP antigen. *Eur J Immunol 27:* 2502–2513.
26. Guerin-Marchand C, Druilhe P, Galey B, Londono A, Patarapotikul J, Beaudoin RL, Dubeaux C, Tartar A, Mercereau-Puijalon O, Langsley G, 1987. A liver-stage-specific antigen of *Plasmodium falciparum* characterized by gene cloning. *Nature 329:* 164–167.
27. Jelinek T, Bluml A, Loscher T, Nothdurft HD, 1998. Assessing the incidence of infection with *Plasmodium falciparum* among international travelers. *Am J Trop Med Hyg 59:* 35–37.