JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1987, p. 91–96
0095-1137/87/010091-06$02.00/0

Vol. 25, No. 1

Detection of Human Antibodies against *Plasmodium falciparum* Sporozoites Using Synthetic Peptides

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Received 19 May 1986/Accepted 2 October 1986

A large peptide consisting of about 40 (Asn-Ala-Asn-Pro) repeats of *Plasmodium falciparum* circumsporozoite protein, $(NANP)_{40}$, was synthesized. It was recognized specifically by monoclonal antibodies produced against *P. falciparum* sporozoites. Moreover, this peptide strongly inhibited the binding of such monoclonal antibodies to antigens present in a sporozoite extract. The $(NANP)_{40}$ peptide was employed without any carrier to develop an enzyme-linked immunosorbent assay to detect sporozoite-specific serum antibodies arising after natural malaria infections. Antibodies were detected in a high percentage (43.1%) of European patients suffering from acute *P. falciparum* malaria and in Africans living in an area of Gabon endemic for malaria. In the latter group, the frequency of antisporozoite antibodies increased with age, reaching 65.9% in individuals more than 40 years old. There was a significant correlation between the results obtained with an immunofluorescence assay with glutaraldehyde-fixed sporozoites and those obtained by enzyme-linked immunosorbent assay with $(NANP)_{40}$. Therefore, such synthetic peptides representing the repetitive epitope of *P. falciparum* circumsporozoite protein can be used for the detection of antisporozoite antibodies and for the epidemiological studies required to obtain base-line data concerning the immune status of individuals before their participation in a sporozoite vaccine trial.

Despite their short persistence in the bloodstream after mosquito bites, sporozoites may play a role in the induction of immune protection against malaria. In fact, evidence exists that irradiated sporozoites can confer stage- and species-specific immunity, both in animals and in humans challenged with living parasites (6). This protection has been related to the ability of the antibodies produced against sporozoites to inhibit their penetration into liver cells (10, 11). By using an immunoradiometric assay, it was found that most polyclonal and monoclonal antibodies (MAbs) reacting with sporozoites recognize a restricted region of the circumsporozoite (CS) protein (29). This region has been shown recently to consist for *Plasmodium falciparum* of four amino acids (Asn-Ala-Asn-Pro) repeated 37 times (7, 9). Moreover, all the *P. falciparum* strains investigated to date exhibit this particular repetitive epitope (26, 30), i.e., there is no strain specificity for this CS protein, unlike the CS proteins of the simian parasites *P. knowlesi* (23) and *P. cynomolgi* (5, 8). Taken together, these data suggest that recombinant or synthetic peptides reproducing the repeat region of the *P. falciparum* CS protein may provide a means to assess the antisporozoite antibody response.

The usual method for the measurement of sporozoite antibodies in both natural and experimental malaria infections has been the immunofluorescence assay (IFA) with living or glutaraldehyde-fixed sporozoites (16). This test requires a large, constant supply of sporozoites which are extracted from infected mosquitoes.

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In the present study, an enzyme-linked immunosorbent assay (ELISA) with a large synthetic peptide consisting of about 40 Asn-Ala-Asn-Pro repeats was developed for the detection of human antibodies against *P. falciparum* sporozoites. This ELISA was evaluated and compared with the classical IFA. The ELISA has been used for the detection of antisporozoite antibodies in individuals living in areas endemic for *P. falciparum* malaria and in Europeans with acute malaria.

MATERIALS AND METHODS

Peptides. Peptides consisting of an average of 4, 20, and 40 Asn-Ala-Asn-Pro repeats of the *P. falciparum* CS protein [$(NANP)_4$, $(NANP)_{20}$, and $(NANP)_{40}$, respectively] were synthesized in the Polypeptide Synthesis Department, Eniricerche, Monterotondo, Italy. The original synthesis of the sequential polytetrapeptides has been described in the Italian Patent Application no. 21718, 25 June 1985, and will be reported in detail in a forthcoming publication. The lyophilized material was dissolved in sterile distilled water at 1 mg/ml, divided into aliquots, and stored at −70°C until use.

Parasites and parasite antigens. *Anopheles stephensi* mosquitoes (a laboratory stock recently derived from wild mosquitoes caught in Pakistan) infected with *P. falciparum* sporozoites (isolate NF 54, Amsterdam Airport strain) were ground in phosphate-buffered saline (PBS), pH 7.2 (30 μl per mosquito), containing 0.1% Nonidet P-40 and the protease inhibitors antipain, leupeptin (25 μg/ml each), and aprotinin (2 U/ml) (all from Sigma Chemical Co., St. Louis, Mo.). After several freeze-thawing cycles, the extracts were di-

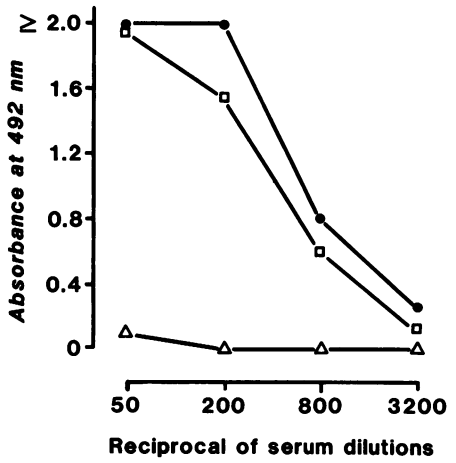

FIG. 1. Measurement of the binding of human antibodies to synthetic peptides. Plates were coated with $(NANP)_{40}$ (●), $(NANP)_{20}$ (□), and $(NANP)_4$ (△) (all at 1 μg/ml) by overnight incubation in a moist chamber. The serum tested was from one adult from Zaire who had suffered several *P. falciparum* malaria attacks.

luted in PBS (120 μl per mosquito) containing 0.25% milk powder and centrifuged at 10,000 × *g* for 20 min, and the supernatants were stored at −20°C. Uninfected mosquitoes served as controls. Extracts of *P. berghei* (ANKA strain)-infected and uninfected *A. stephensi* salivary glands (kindly provided by Bruno Betschart, Swiss Tropical Institute, Basel, Switzerland) were also prepared in the same manner.

Asynchronous cultures of *P. falciparum* isolate FCQ-27 (from Papua New Guinea) were harvested, centrifuged at 1,500 × *g* for 10 min, and washed three times with cold PBS. The cell pellets were treated at room temperature in 20 times their volume of 1% Nonidet P-40 in 50 mM Tris-buffered saline–0.5 mM EDTA, pH 7.4, containing a cocktail of protease inhibitors: 0.2 mM tosyl-phenylchloromethylketone, 1 mM 1,10-phenoanthroline, 2 mM tosyllysylchloromethylketone (Sigma), and 2 mM phenylmethylsulfonylfluoride (Serva, Heidelberg, Federal Republic of Germany). The extracts were centrifuged at 400 × *g* for 10 min, and the supernatants were stored at −20°C.

MAbs. MAbs 2A10 (immunoglobulin G [IgG]2a [18], kindly provided by F. Zavala, New York University, New York) and 3Sp2 (IgG1, provided by J.-P. Verhave) are specific for the CS protein of *P. falciparum*. MAb 3D11 (provided by F. Zavala) is an IgG1k (21) specific for the CS protein of *P. berghei* (27). MAb Hb31cl3 (20; kindly provided by L. Perrin, Blood Transfusion Center, Cantonal Hospital, Geneva, Switzerland) is an IgG recognizing an 82-41-kilodalton antigen present on *P. falciparum* merozoites. MAb 2A10 was used as affinity-purified protein; the other MAbs were used as 50% ammonium sulfate-precipitated ascitic fluids.

ELISA with poly-(NANP) peptides. Flat-bottom 96-well plates (Nunc-Immunoplate I; Nunc, Roskilde, Denmark) were coated by overnight incubation at room temperature in a moist chamber with 100 μl of $(NANP)_{40}$, $(NANP)_{20}$, or $(NANP)_4$ per well, at 10 or 1 μg/ml in PBS, pH 7.8. After four washes with PBS–0.05% Tween 20 (PBS-T), plates were saturated for 1 h at room temperature with 200 μl of PBS-T containing 5% milk powder (14). Then, 100 μl of serum dilutions in PBS-T containing 2.5% milk powder were added to duplicate wells and incubated for 1 h at room temperature. After four washes with PBS-T, 100 μl of horseradish peroxidase-conjugated goat IgG fraction of anti-human IgG, IgA, and IgM (Cooper Biomedical, Inc., West Chester, Pa.) diluted 1:5,000 in PBS-T containing 2.5% milk powder was added per well, and the plates were incubated for 1 h at room temperature. After four washes with PBS-T, 100 μl of the substrate solution (*ortho*-phenylenediamine [Fluka AG, Buchs, Switzerland], 0.4 mg/ml in 0.1 M citrate buffer, pH 5.0, containing 0.01% hydrogen peroxide) was added. The enzymatic reaction was stopped 20 min later by adding 50 μl of 2.5 N H_2SO_4. The optical density (OD) was read in A_{492} with a Titertek Multiskan reader (Flow Laboratories, Inc., McLean, Va.). In each assay, uncoated plates were used, incubated with PBS (pH 7.8) and then processed in the same way as for the coated plates. Results were expressed as the difference between the OD values obtained in coated and uncoated plates.

In some experiments, coated plates were stored at 4°C in a moist chamber and then tested up to 5 weeks later. Results were compared with those obtained with plates coated the night before the test.

Binding of sporozoite-specific MAbs to solid-phase $(NANP)_{40}$. Plates were coated with $(NANP)_{40}$ (1μg/ml), with extracts of *P. falciparum*- and *P. berghei*-infected mosquitoes (diluted 1:100), or with extracts of *P. falciparum* erythrocytic forms (FCQ-27, 200 μg/ml). Then, different dilutions of 2A10, 3Sp2, 3D11, and Hb31cl3 MAbs were dispensed in triplicate, and the binding to the different antigens was tested in an ELISA as described above, with two major changes: (i) all incubations were for 2 h; (ii) alkaline phosphatase-conjugated (2) rabbit IgG fraction anti-mouse IgG, diluted 1:100, was used. The enzymatic reaction was revealed by adding as a substrate *p*-nitrophenyl phosphate (Sigma) at 1 mg/ml in 0.01 M diethanolamine solution. The results were read at 405 nm.

ELISA competition assay. Plates were coated with an extract of *P. falciparum* sporozoites (1:100). Separately, fixed concentrations of 2A10 and 3Sp2 MAbs were incubated for 1 h at 37°C with different 10-fold dilutions of synthetic peptides in PBS-T containing 2.5% milk powder. Then, 100 μl of the single mixtures was added to triplicate precoated wells, and the ELISA was carried out as described above. As a control, coated wells received MAbs alone.

Human sera. Sera were obtained from: (i) 406 children and adults living in Okondja, Gabon, an area endemic for *P. falciparum* malaria; (ii) adult Europeans with acute *P. falciparum* or *P. vivax* malaria or with other parasitic diseases; (iii) 17 adult Europeans with tuberculosis; (iv) 388 healthy blood donors living in Geneva (Blood Transfusion Center, Cantonal Hospital, Geneva, Switzerland).

IFA for anti-*P. falciparum* sporozoite antibodies. An IFA for anti-*P. falciparum* sporozoite antibodies was done in Nijmegen by using glutaraldehyde-fixed sporozoites as previously described (16). Sera with a titer of ≥1:10 were considered positive. As an additional confirmation, some sera were reexamined with the IFA by using suspensions of living, freshly isolated sporozoites as the antigen.

RESULTS

Selection of peptides suitable for use in a solid-phase ELISA. Three polypeptides reproducing the amino acid sequence of the repetitive epitope of the CS protein of *P. falciparum* were tested to determine which one(s) could be used efficiently for an ELISA to detect anti-CS protein antibodies. Different concentrations of $(NANP)_{40}$, $(NANP)_{20}$, and $(NANP)_4$ were used (without coupling to carrier proteins) to

TABLE 1. Specific recognition of $(NANP)_{40}$ synthetic peptide by anti-*P. falciparum* CS protein-specific monoclonal antibodies

Plates coated with:	Results[a] for:			
	Anti-*P. falciparum* MAb		Anti-*P. berghei* MAb 3D11[d]	Anti-*P. falciparum* merozoite MAb Hb31c13[d]
	2A10[b]	3Sp2[c]		
$(NANP)_{40}$ (1 μg/ml)	>2	>2	0.01 ± 0.01	0.03 ± 0.01
Mosquito extracts (1:100)				
P. falciparum-infected *A. stephensi* mosquitoes	1.92 ± 0.01	1.09 ± 0.02	0.01 ± 0.01	0.06 ± 0.01
P. berghei-infected *A. stephensi* salivary glands	0	ND[e]	1.20 ± 0.02	ND
Noninfected *A. stephensi* mosquitoes	0	0	0	0.05 ± 0.01
Extract of *P. falciparum* erythrocytic forms (FCQ-27 isolate) (200 μg/ml)	ND	0.03 ± 0.01	ND	>2

[a] Results expressed as OD at 405 nm; mean of triplicate wells ± the standard deviation.
[b] 5 μg/ml.
[c] 7 μg/ml.
[d] 50 μg/ml.
[e] ND, Not done.

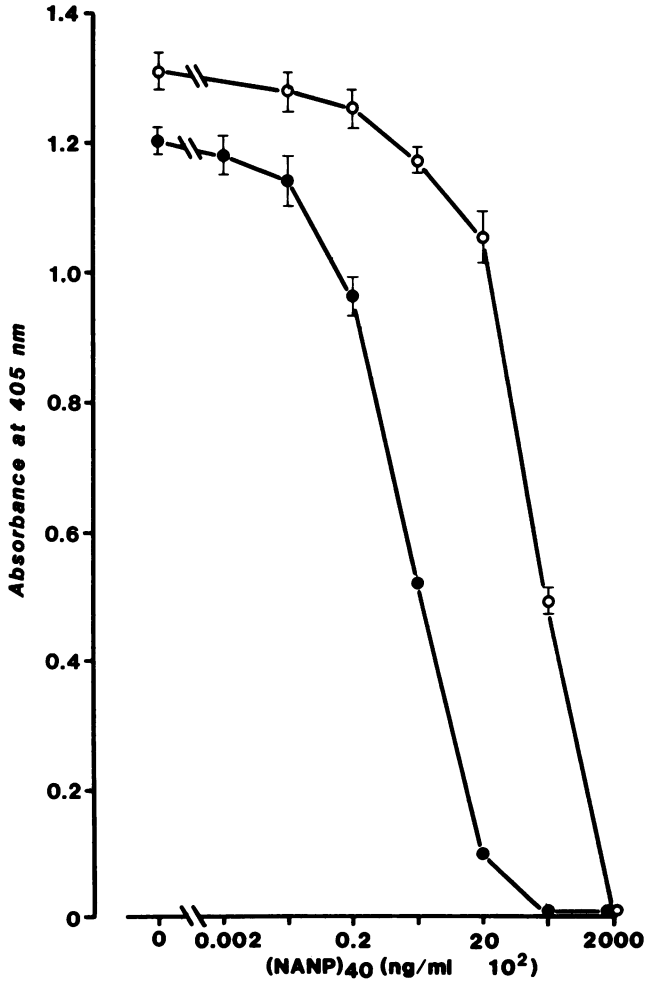

FIG. 2. $(NANP)_{40}$ peptide inhibition of the binding of MAbs to an extract of *P. falciparum* sporozoites. 2A10 (50 ng/ml) (●) and 3Sp2 (3.5 μg/ml) (○) were incubated for 1 h at 37°C with different concentrations of $(NANP)_{40}$. The mixtures were then reacted with *P. falciparum* sporozoite extract-coated plates. Each point represents the mean of triplicate wells ± the standard deviation (not represented when <0.01).

coat microwells, and an ELISA was performed with human sera containing antibodies to *P. falciparum* sporozoites. At 1 μg/ml, the most efficient antibody binding was obtained with $(NANP)_{40}$, whereas the reaction was negative with $(NANP)_4$ at that concentration (Fig. 1). At 10 μg/ml, comparable results were obtained with $(NANP)_{40}$ and $(NANP)_{20}$, but the binding level was considerably lower with $(NANP)_4$ (data not shown). Thus, $(NANP)_{40}$ at 1 μg/ml was selected for coating the wells in all subsequent tests. Plates coated with $(NANP)_{40}$ up to 5 weeks before the test and stored at 4°C in a moist chamber were found to yield reproducible results.

Specific recognition of solid-phase $(NANP)_{40}$ by *P. falciparum* CS protein-specific MAbs. Two MAbs specific for *P. falciparum* CS protein (2A10 and 3Sp2), one specific for *P. berghei* CS protein (3D11), and one specific for *P. falciparum* merozoites (Hb31cl3) were tested in ELISA by using wells coated with $(NANP)_{40}$, extracts of *P. falciparum* and *P. berghei* sporozoites, or an extract of *P. falciparum* erythrocytic forms. The results obtained in two such experiments are summarized in Table 1. $(NANP)_{40}$ was recognized specifically by 2A10 and 3Sp2 and not by 3D11 or Hb31cl3. The latter two MAbs did recognize extracts of *P. berghei* sporozoites (3D11) and *P. falciparum* erythrocytic forms (Hb31cl3), respectively.

Synthetic (NANP) polymers inhibit the binding of sporozoite-specific MAbs to the repetitive epitope of *P. falciparum* CS protein. To answer the question of whether the repetitive epitope of the *P. falciparum* CS protein was represented by the synthetic peptides, a competition ELISA was carried out. Fixed concentrations of 2A10 and 3Sp2 MAbs (50 ng/ml and 3.5 μg/ml, respectively) were incubated for 1 h at 37°C with different concentrations of $(NANP)_{40}$, $(NANP)_{20}$, and $(NANP)_4$. The mixtures were then reacted in plates coated with an extract of *P. falciparum*-infected mosquitoes. $(NANP)_{40}$ was highly effective in inhibiting the binding of both MAbs to the mosquito extract: less than 0.2 μg of peptide per ml gave 50% inhibition of the binding of 2A10 MAb (Fig. 2). With $(NANP)_{20}$ and $(NANP)_4$, complete inhibition was also achieved, but it required a higher molar concentration (data not shown). It should be noted that $(NANP)_{40}$ did not inhibit the binding of 3D11 MAb to an extract of *P. berghei*-infected *A. stephensi* salivary glands at comparable concentrations tested.

Comparison between $(NANP)_{40}$ ELISA and IFA results. Coded serum samples (64) were tested in a double-blind study by IFA in Nijmegen and by ELISA in Geneva. There was a statistically significant correlation between the results obtained in the IFA with fixed sporozoites and those ob-

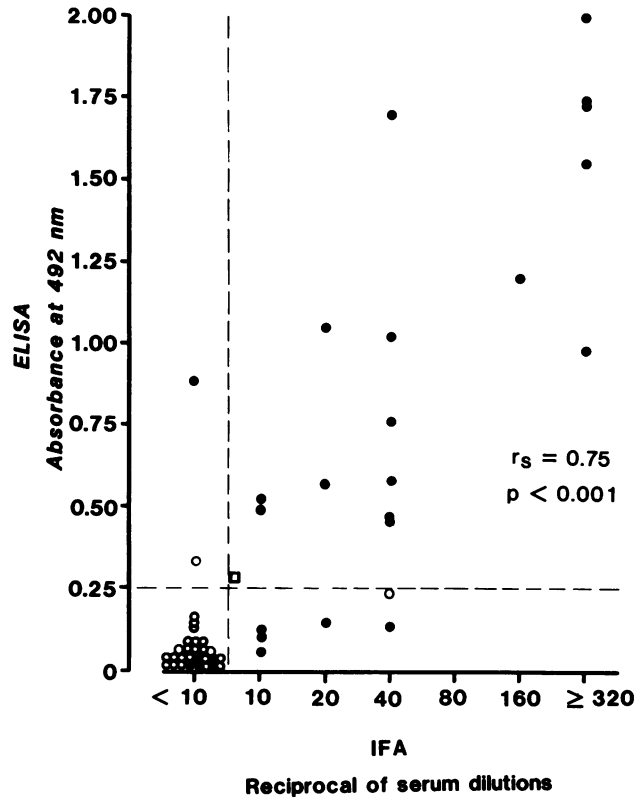

FIG. 3. Comparison between $(NANP)_{40}$ ELISA and IFA with glutaraldehyde-fixed *P. falciparum* sporozoites for the detection of antisporozoite antibodies in human sera. Closed circles (●) represent single individuals with *P. falciparum* malaria; most of them were European travelers returning with acute malaria from endemic countries. Open circles (○) represent single healthy blood donors or normal laboratory volunteers who never had acute malaria. The open square (□) identifies the serum from one blood donor which was positive in IFA when tested undiluted but negative when diluted 1:5. Dotted lines represent the upper limit of normal values for ELISA (98th percentile) and IFA (<1:10).

tained in the ELISA (Spearman rank correlation: $r_s = 0.75$, $P < 0.001$) (Fig. 3). Three patients with smear-positive *P. vivax* malaria were included; two were positive in IFA and in ELISA. In addition, one of three patients with filariasis was positive in ELISA, but all three were negative in IFA. Finally, one patient with amoebiasis was negative in both tests. Based on the positive/negative criterion, both tests scored identically in 87.5% of the sera; 44% were found positive in the IFA, and 41% were positive in the ELISA. IFA results obtained with live sporozoites were in line with the above mentioned findings.

Detection of human antisporozoite antibodies by the $(NANP)_{40}$ ELISA. Different groups of human sera were then tested by ELISA. The results were compared with those obtained by testing sera from 388 healthy blood donors. The 98th percentile of OD values at 492 nm in sera diluted 1:200 was chosen as the upper limit of the normal range (0.25 OD).

Of 51 Europeans with acute *P. falciparum* malaria, 22 (43.1%) were positive (OD, >0.25) for anti-$(NANP)_{40}$ antibodies, whereas none of the 17 Europeans with culture-positive tuberculosis gave positive results. In a preliminary study, the frequency of individuals positive for such antibodies was analyzed in areas endemic for *P. falciparum* malaria. Antibodies to $(NANP)_{40}$ were present in 42.6% of 406 individuals living in Okondja, Gabon. The frequency of positive subjects increased with age, reaching 65.9% in individuals more than 40 years old (Table 2).

DISCUSSION

Recently, peptides have been synthesized reproducing the repetitive epitopes of *P. knowlesi* (22), *P. falciparum* (3, 7, 31), and *P. vivax* (1, 15) CS proteins, as well as the group-specific highly conserved regions I and II of CS proteins (3, 7, 25). The natural epitopes are well conserved in these CS synthetic peptides and, thus, they represent substitutes for the natural antigen which are now under consideration as potential vaccines (19, 31).

In this paper, we demonstrated that the synthetic peptide $(NANP)_{40}$ reproducing the repetitive epitope of *P. falciparum* CS protein can be used in a solid-phase immunoassay to detect antisporozoite antibodies occurring after natural malaria infections. The large size of this peptide renders it suitable for a direct coating of microwells. A level of 100 ng per well was sufficient to detect efficiently the presence of antibodies in sera (Fig. 1). The smaller the peptide, the higher the concentration required to achieve comparable results. It is noteworthy that the coupling of the peptide(s) to carrier proteins is not needed. In studies published to date with sporozoite synthetic peptides, smaller peptides (3 to 4 repeats) had to be coupled to a carrier to allow the detection of antibodies raised in animals by immunization with these peptides (3, 28, 31). Avoiding the use of carriers for immunization and detection may offer certain advantages. Indeed, the coupling reaction may alter the activity of the antigenic determinant present on the peptide, depending on the residues involved in the coupling (4). $(NANP)_{40}$ peptide can be used for coating at a very low concentration (1 μg/ml), and 1 mg suffices to test more than 3,500 serum samples. In addition, plates could be coated with this peptide up to 1 month before the test, without any appreciable variation in results.

In agreement with the results obtained by using different synthetic peptides of the repetitive epitope of *P. falciparum* CS protein (7, 31), $(NANP)_{40}$ peptide was recognized specifically only by MAbs directed against *P. falciparum* sporozoites. Moreover, this peptide strongly inhibited the binding of such MAbs to an extract of sporozoites (Fig. 2). Smaller peptides $(NANP)_{20}$ and $(NANP)_4$, although good inhibitors, were less active on a molar basis. The conformation of the large polypeptide molecule may be more similar than that of the small peptides to the conformation of the native protein and may react with a higher avidity with antibody molecules.

TABLE 2. Detection of human antisporozoite antibodies by use of the $(NANP)_{40}$ ELISA

Subjects	Age (yrs)	No. positive/ no. tested (%)
European patients with:		
Acute *P. falciparum* malaria	Adults	22/51 (43.1)
Tuberculosis	Adults	0/17 (0.0)
African individuals from endemic area	<2	1/24 (4.2)
(Okondja, Gabon)	2–10	16/72 (22.2)
	11–20	63/161 (39.1)
	21–30	15/26 (57.7)
	31–40	16/29 (55.2)
	>40	62/94 (65.9)

The use of the $(NANP)_{40}$ peptide has led to the development of an ELISA to detect *P. falciparum* sporozoite antibodies present in humans after natural infection. Such antibodies were found in a high percentage (43.1%) of European adults who had one acute malaria attack upon returning from countries endemic for *P. falciparum* malaria. Further studies are required to exclude the possibility that a patent parasitemia augments the percentage of positive test results, either by polyclonal activation or the presence of antigenic determinants shared by sporozoites and asexual blood forms (12). However, in a recent report, Zavala et al. (32) have shown that naturally occurring antisporozoite antibodies do not cross-react with the synthetic Ag5.1 protein, despite the presence of a NANP sequence in the Ag5.1 protein (13) and despite the cross-reactivity of some monoclonal antibodies (12). Our preliminary studies indicate that the frequency of antisporozoite antibodies in individuals living in an endemic area in Gabon increased with age, reaching 65.9% in adults more than 40 years old. These results confirm those obtained by IFA in populations living in endemic areas of Gambia (17), Thailand (24) and Kenya (J.-P. Verhave, unpublished observations), and by immunoradiometric assay also testing sera from Gambia (19; F. Zavala, J. P. Tam, R. S. Nussenzweig and V. Nussenzweig, Fed. Proc. **44**:980, 1985) and Kenya (J.-P. Verhave, unpublished observations). Obviously, the various values obtained in these studies are related to the different levels of malaria endemicity in the locations chosen for study.

Recently, an immunoradiometric assay with a smaller $(NANP)_3$ CS synthetic peptide has been used to detect antisporozoite antibodies in sera from individuals living in Gambia (19; Zavala et al., Fed. Proc., 1985), yielding results similar to those obtained by IFA. In the present study, when serum samples were tested both by IFA on glutaraldehyde-fixed or live sporozoites and by ELISA with $(NANP)_{40}$ peptide, a significant correlation was observed between the results obtained with these two methods. This correlation confirms that the $(NANP)_{40}$ ELISA can be used to detect anti-*P. falciparum* sporozoite antibodies. The ELISA results were recently confirmed by a similar test with the recombinant *P. falciparum* CS protein R32tet32 (28). Both ELISA methods yielded corresponding positive and negative results for the majority of sera tested (unpublished observations).

This ELISA should prove to be useful for the measurement of antisporozoite antibodies, particularly in epidemiological studies. Indeed, this assay does not require any source of sporozoites, beyond the present technical evaluation; it can be carried out simply and rapidly with a low peptide concentration; it does not require any radioactive reagents, and the coated plates have been shown to remain stable for several weeks at 4°C. It can be applied easily to the evaluation of the spontaneous antisporozoite antibody response in populations exposed to natural malaria infections. Also, it can provide information on the immune status of individuals in which vaccination trials would be undertaken in the future by using sporozoite antigens obtained by synthetic (3, 19, 31) or recombinant DNA (28) techniques.

ACKNOWLEDGMENTS

We thank F. Zavala, L. Perrin, and B. Betschart for their generous gifts of reagents and F. Speiser (Swiss Tropical Institute), M. Van Hoegarden, and A. Dupont (Centre Internationale de Recherche Médicale de Franceville) for making available characterized human serum samples. We also thank J. A. Cooper (World Health Organization Immunology Research and Training Centre, Geneva), A. D. E. M. Leeuwenberg (Department of Medical Parasitology, Nijmegen), G. Gnocchi and S. Squadrini (Eniricerche, Monterotondo) for technical assistance, and M. Devouge for typing the manuscript.

This study was supported by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases, by the Lord Michelham of Hellingly Foundation, and by the Swiss National Foundation (grant no. 3.826.0.83). G.D.G. was partially supported by an award from the Ministero della Pubblica Istruzione, Italy.

LITERATURE CITED

1. **Arnot, D. E., J. W. Barnwell, J. P. Tam, V. Nussenzweig, R. S. Nussenzweig, and V. Enea.** 1985. Circumsporozoite protein of *Plasmodium vivax:* gene cloning and characterization of the immunodominant epitope. Science **230**:815–818.
2. **Avrameas, S.** 1969. Coupling of enzymes to proteins with glutaraldehyde: use of the conjugates for the detection of antigens and antibodies. Immunochemistry **6**:43–52.
3. **Ballou, W. R., J. Rothbard, R. A. Wirtz, D. M. Gordon, J. L. Williams, R. W. Gore, I. Schneider, M. R. Hollingdale, R. L. Beaudoin, W. L. Maloy, L. H. Miller, and W. T. Hockmeyer.** 1985. Immunogenicity of synthetic peptides from circumsporozoite protein of *Plasmodium falciparum*. Science **228**: 996–999.
4. **Briand, J. P., S. Muller, and M. H. V. Van Regenmortel.** 1985. Synthetic peptides as antigens: pitfalls of conjugation methods. J. Immunol. Methods **78**:59–69.
5. **Cochrane, A. H., R. W. Gwadz, E. Ojo-Amaize, J. Hii, V. Nussenzweig, and R. S. Nussenzweig.** 1985. Antigenic diversity of the circumsporozoite proteins in the *Plasmodium cynomolgi* complex. Mol. Biochem. Parasitol. **14**:111–124.
6. **Cochrane, A. H., R. S. Nussenzweig, and E. H. Nardin.** 1980. Immunization against sporozoites, p. 163–202. *In* J. P. Kreier (ed.), Malaria, vol. 3. Academic Press, Inc., New York.
7. **Dame, J. B., J. L. Williams, T. F. McCutchan, J. L. Weber, R. A. Wirtz, W. T. Hockmeyer, W. L. Maloy, J. D. Haynes, I. Schneider, D. Roberts, G. S. Sanders, E. P. Reddy, C. L. Diggs, and L. H. Miller.** 1984. Structure of the gene encoding the immunodominant surface antigen on the sporozoite of the human malaria parasite *Plasmodium falciparum*. Science **225**:593–599.
8. **Enea, V., D. Arnot, E. C. Schmidt, A. H. Cochrane, R. W. Gwadz, and R. S. Nussenzweig.** 1984. Circumsporozoite gene of *Plasmodium cynomolgi* (Gombak): cDNA cloning and expression of the repetitive circumsporozoite epitope. Proc. Natl. Acad. Sci. USA **81**:7520–7524.
9. **Enea, V., J. Ellis, F. Zavala, D. E. Arnot, A. Asanavich, A. Masuda, I. Quakyi, and R. S. Nussenzweig.** 1984. DNA cloning of *Plasmodium falciparum* circumsporozoite gene: amino acid sequence of repetitive epitope. Science **225**:628–630.
10. **Hollingdale, M. R., E. H. Nardin, S. Tharavanij, A. L. Schwartz, and R. S. Nussenzweig.** 1984. Inhibition of entry of *Plasmodium falciparum* and *P. vivax* sporozoites into cultured cells: an *in vitro* assay of protective antibodies. J. Immunol. **132**:909–913.
11. **Hollingdale, M. R., F. Zavala, R. S. Nussenzweig, and V. Nussensweig.** 1982. Antibodies to the protective antigen of *Plasmodium berghei* sporozoites prevent entry into cultured cells. J. Immunol. **128**:1929–1930.
12. **Hope, I. A., R. Hall, D. L. Simmons, J. E. Hyde, and J. G. Scaife.** 1984. Evidence for immunological cross reaction between sporozoites and blood stages of a human malaria parasite. Nature (London) **308**:191–194.
13. **Hope, I. A., M. Mackay, J. E. Hyde, M. Goman, and J. Scaife.** 1985. The gene for an exported antigen of the malaria parasite *Plasmodium falciparum* cloned and expressed in *E. coli*. Nucleic Acids Res. **13**:369–379.
14. **Johnson, D. A., J. W. Gautsch, J. R. Sportsman, and J. H. Elder.** 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. Gene Anal. Technol. **1**:3–8.
15. **McCutchan, T. F., A. A. Lal, V. F. De La Cruz, L. H. Miller,**

W. L. Maloy, Y. Charoenvit, R. L. Beaudoin, P. Guerry, R. Wistar, Jr., S. L. Hoffman, W. T. Hockmeyer, W. E. Collins, and D. Wirth. 1985. Sequence of the immunodominant epitope for the surface protein on sporozoites of *Plasmodium vivax*. Science **230**:1381–1383.

16. **Nardin, E. H., R. W. Gwadz, and R. S. Nussenzweig.** 1979. Characterization of sporozoite surface antigens by indirect immunofluorescence: detection of stage- and species-specific antimalarial antibodies. Bull. W.H.O. **57**(Suppl. 1):211–217.
17. **Nardin, E. H., R. S. Nussenzweig, I. A. McGregor, and J. H. Bryan.** 1979. Antibodies to sporozoites: their frequent occurrence in individuals living in an area of hyperendemic malaria. Science **206**:597–599.
18. **Nardin, E. H., V. Nussenzweig, R. S. Nussenzweig, W. E. Collins, K. Harinasuta, P. Tapchaisri, and Y. Chomcharn.** 1982. Circumsporozoite proteins of human malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*. J. Exp. Med. **156**: 20–30.
19. **Nussenzweig, V., and R. S. Nussenzweig.** 1985. Malaria vaccine against sporozoites? Ann. Inst. Pasteur (Paris) **136**:301–312.
20. **Perrin, L. H., and R. Dayal.** 1982. Immunity to asexual erythrocytic stages of *Plasmodium falciparum:* role of defined antigens in the humoral response. Immunol. Rev. **61**:245–269.
21. **Potocnjak, P., N. Yoshida, R. S. Nussenzweig, and V. Nussenzweig.** 1980. Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection. J. Exp. Med. **151**:1504–1513.
22. **Schlesinger, D. H., A. H. Cochrane, R. W. Gwadz, G. N. Godson, R. Melton, R. S. Nussenzweig, and V. Nussenzweig.** 1984. Structure of an immunodominant epitope of the circumsporozoite surface protein of *Plasmodium knowlesi*. Biochemistry **23**:5665–5670.
23. **Sharma, S., P. Svec, G. H. Mitchell, and G. N. Godson.** 1985. Diversity of circumsporozoite antigen genes from two strains of the malarial parasite *Plasmodium knowlesi*. Science **229**: 779–782.
24. **Tapchaisri, P., Y. Chomcharn, C. Poonthong, A. Asavanich, S. Limsuwan, O. Maleevan, S. Tharavanij, and T. Harinasuta.** 1983. Anti-sporozoite antibodies induced by natural infection. Am. J. Trop. Med. Hyg. **32**:1203–1208.
25. **Vergara, U., A. Ruiz, A. Ferreira, R. S. Nussenzweig, and V. Nussenzweig.** 1985. Conserved group-specific epitopes of the circumsporozoite proteins revealed by antibodies to synthetic peptides. J. Immunol. **134**:3445–3448.
26. **Weber, J. L., and W. T. Hockmeyer.** 1985. Structure of the circumsporozoite protein in 18 strains of *Plasmodium falciparum*. Mol. Biochem. Parasitol. **15**:305–316.
27. **Yoshida, N., R. S. Nussensweig, P. Potocnjak, V. Nussenzweig, and M. Aikawa.** 1980. Hybridoma produces protective antibodies directed against the sporozoite stage of malaria parasite. Science **207**:71–73.
28. **Young, J. F., W. T. Hockmeyer, M. Gross, W. R. Ballou, R. A. Wirtz, J. H. Trosper, R. L. Beaudoin, M. R. Hollingdale, L. H. Miller, C. L. Diggs, and M. Rosenberg.** 1985. Expression of *Plasmodium falciparum* circumsporozoite proteins in *Escherichia coli* for potential use in a human malaria vaccine. Science **228**:958–962.
29. **Zavala, F., A. H. Cochrane, E. H. Nardin, R. S. Nussenzweig, and V. Nussenzweig.** 1983. Circumsporozoite proteins of malaria parasites contain a single immunodominant region with two or more identical epitopes. J. Exp. Med. **157**:1947–1957.
30. **Zavala, F., A. Masuda, P. M. Graves, V. Nussenzweig, and R. S. Nussenzweig.** 1985. Ubiquity of the repetitive epitope of the CS protein in different isolates of human malaria parasites. J. Immunol. **135**:2790–2793.
31. **Zavala, F., J. P. Tam, M. R. Hollingdale, A. H. Cochrane, I. Quakyi, R. S. Nussenzweig, and V. Nussenzweig.** 1985. Rationale for development of a synthetic vaccine against *Plasmodium falciparum* malaria. Science **228**:1436–1440.
32. **Zavala, F., J. P. Tam, and A. Masuda.** 1986. Synthetic peptides for the detection of humoral immunity to *Plasmodium falciparum* sporozoites. J. Immunol. Methods **93**:55–61.