

Defined *Plasmodium falciparum* antigens in malaria serology

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The study evaluates three enzyme-linked immunosorbent assays (ELISA) of malaria antigens suitable for use in large-scale epidemiological studies. Results obtained using sera from 567 persons from the Gambia indicated that the micro-ELISA method using parasitized red blood cell extract did not reliably quantitate antimalarial antibodies, especially in young children. In contrast, two micro-ELISA methods that employed purified, defined antigens (a polypeptide of $M_r=41\ 000$ present in rhoptries, and a 31-1 fusion polypeptide corresponding to a merozoite surface antigen) permitted the precise determination of antimalarial antibodies in both adults and children. Problems and advantages associated with the use of the $M_r=41\ 000$ and 31-1 antigens for the determination of antimalarial antibodies are discussed.

There is a need for improved and cheap immuno-diagnostic tests for the detection of antimalarial antibodies (1). In areas where malaria is endemic such tests could be used for epidemiological studies (2-4) and, in future, to evaluate malaria vaccines; furthermore, the tests could also be employed to screen blood donors returning from areas where the disease is endemic.

Various methods have been used to detect antimalarial antibodies, including gel precipitation (5), indirect haemagglutination (6), indirect immunofluorescence (7), radioimmunoassays (RIA), and enzyme-linked immunosorbent assays (ELISA) (8, 9). RIA and ELISA are being used increasingly for epidemiological studies because they combine sensitivity with low cost and are amenable to automation; however, their main limitation has been the lack of standardized reagents, especially antigens. The principal source of malaria has been blood from infected individuals or experimental animals and, more recently, *Plasmodium falciparum* asexual blood stages from *in vitro* cultures (3, 9, 11). Never-

theless, extracts prepared from parasitized erythrocytes contain a wide range of antigens, including normal red blood cell (NRBC) components, and their production and purification are not standardized. In addition, the presence of NRBC components in the extracts may present an important limitation, since sera of individuals from areas where malaria is endemic frequently contain high levels of anti-NRBC antibodies (10, 11).

In the present study, the following antigen sources were evaluated using a micro-ELISA method to detect antimalarial antibody: *P. falciparum*-infected red blood cell extract, NRBC extract as control, and two merozoite-purified components—a polypeptide of relative molecular mass (M_r) 41 000 associated with the rhoptries and a polypeptide corresponding to part of the main merozoite surface component (pMc31-1).

MATERIALS AND METHODS

Plasma and sera

Samples of plasma from 567 inhabitants of the village of Kenaba in the Gambia were collected during a survey conducted in 1970. The following information was recorded for each participant: age, sex, and an estimate of the level of parasitaemia. The samples were stored frozen at $-20\ ^\circ\text{C}$ in small aliquots and sera were recovered after defibrination (14). Samples of sera from two adults in Côte

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d'Ivoire and from five adults in Colombia who had had frequent malaria infections formed a pool of positive sera. Sera from the following groups served as controls: 15 samples from Swiss patients with acute bacterial infection; 12 samples from Brazilian patients with schistosomiasis; and 50 samples from healthy blood donors who had never visited malaria endemic areas.

Antigens for micro-ELISA tests

Parasitized red blood cells (PRBC). The SGE2 isolate of *P. falciparum* was cultured *in vitro* for 3 years and then cultivated in 100-mm Petri dishes (12). The PRBC, which were collected by centrifugation when the parasitaemia level reached 15%, were washed three times in 20 volumes of phosphate-buffer saline (PBS; pH 7.4) and a washed pellet (2 ml) was then dissolved in 20 ml of lysis buffer (PBS containing 1% Nonidet P-40, 0.2% Tween 20, 5 mmol/l ethylenediaminetetraacetic acid) and 2 mmol/l phenylmethylsulfonyl fluoride). The lysate was centrifuged at 2000 g for 15 minutes and the supernatant that was collected was divided into aliquots, and stored at -75°C .

Normal red blood cells (NRBC). An extract of normal red blood cells was prepared using the procedure described for the preparation of PRBC.

Polypeptide ($M_r=41\ 000$). The purification of the schizont- and merozoite-specific polypeptide of $M_r=41\ 000$ has been described previously in detail (2).

31-1 Fusion polypeptide. The pMc31-1 plasmid was isolated from a complementary DNA library constructed from mRNA purified from asexual blood stages of SGE2 (13). The 31-1 fusion protein coded by the pMc31-1 plasmid contains a fragment of a schizont- and merozoite-specific polypeptide of $M_r=190\ 000$ -200 000, and at least some of its epitopes are expressed on a merozoite surface component of $M_r=83\ 000$, a processed product of a $M_r=190\ 000$ -200 000 polypeptide (15). The 31-1 fusion polypeptide represents approximately 15% of the protein content of an *Escherichia coli* lysate containing the pMc31-1 plasmid. The polypeptide isolate was enriched by up to 50% by collecting the *E. coli* membrane after treatment of the washed *E. coli* pellet with a solution containing 8 mol/l urea and 0.5% sodium dodecyl sulfate. The membrane extract was applied on 8% sodium dodecyl sulfate-polyacrylamide gel, electrophoresed, and the band containing the 31-1 fusion polypeptide was recovered by electro-elution (12). The final preparation had a protein concentration of 300 $\mu\text{g/ml}$, and SDS-PAGE analysis revealed a single band of $M_r=23\ 000$.

Assay procedure

The micro-ELISA was performed using Linbro flat-bottom polystyrene micro-ELISA plates.^a The plates were coated overnight at 4°C with 100 μl of the optimum concentration of each of the four antigenic solutions (except for the NRBC extract, which was used at the same protein concentration as the PRBC extract); the antigenic preparations were diluted in 0.1 mol/l bicarbonate buffer (pH 9). For the $M_r=41\ 000$ and the 31-1 polypeptides, the bicarbonate buffer was supplemented with a 1 mg/l solution of radioimmunoassay-grade bovine serum albumin (BSA).^b The optimum concentrations of each antigenic preparation, determined by a checkerboard titration using positive and negative control sera containing known concentrations of antigen, were as follows: 10 μg per well for PRBC and NRBC extracts (dilution 1:1500); 10 ng per well for the $M_r=41\ 000$ polypeptide; and 30 ng per well for the 31-1 polypeptide. After incubation with the antigens, the plates were washed three times with PBS supplemented with 0.1% BSA (w/v) and 0.4% Tween 20 (PBS/BSA/Tween) and then incubated with 100 μl of 1% BSA in PBS for 1 hour at room temperature to prevent non-specific binding to the plastic. Subsequently, plates were washed as described above and 0.1 ml of the various sera diluted in PBS/BSA/Tween added to the wells. The plates were then incubated for 3 hours at room temperature, washed six times with PBS/BSA/Tween, and 0.1 ml of goat anti-human total immunoglobulin (λ - and K-light-chain specific) that had been affinity-purified and conjugated with alkaline phosphatase^c was added to each well. After further 2 hours' incubation at room temperature, the plates were washed six times, and 100 μl of *p*-nitrophenyl phosphate^b diluted in enzyme substrate buffer (10% diethanolamine, 0.5 mmol/l magnesium chloride, pH 9.8) added to each well. Finally, the plates were incubated in the dark for either about 2 hours (PRBC antigen, NRBC extract, and 31-1 polypeptide) or about 5 hours ($M_r=41\ 000$ polypeptide). In order to minimize inter-test variations, the precise incubation time was varied depending on the reaction of a pool of positive sera tested at three dilutions (1:100, 1:400, and 1:2000). Additional controls on each plate included one serum weakly positive for malaria antibodies (tested at a dilution of 1:400) and two sera from normal blood donors (tested at a dilution of 1:100). All samples of sera from patients were assayed in duplicate at dilutions of 1:100 and 1:2000. The absorbance at

^a Flow Laboratories, Irvine, Scotland.

^b Sigma Chemical Co., St. Louis, MO, USA.

^c Cappel Laboratories, West Chester, PA, USA.

$\lambda = 405$ nm of the contents of each well was measured using a multiscan photometer.^d

Binding inhibition experiments

Two samples of sera from patients from Côte d'Ivoire with high titres of antimalarial antibody were incubated with each of the four antigens used in the micro-ELISA test (one volume of serum diluted 1:50 plus one volume of the antigenic solution diluted in PBS) to obtain in the mixture a concentration of antigens per 100 μ l corresponding to 1 \times , 10 \times , or 100 \times that of the antigens used to coat the plates. The mixtures thus obtained were incubated overnight, centrifuged at 3000 g for 15 minutes, and the supernatants used in the micro-ELISA tests.

Statistical analysis. The results were analysed by computer using the Statistical Package for the Social Sciences.^e Correlation coefficients were calculated using Pearson's test for pairs of variables.

RESULTS

The results obtained for patients from areas where malaria is endemic were analysed as a function of age in order to compare the data for adolescents, adults, and children. For each age group, the number of patients, the proportion with patent parasitaemia (defined as the presence of asexual and/or sexual blood stages of either *P. falciparum*, *P. ovale*, or *P. malariae* parasites on thick blood smears), together with the haemoglobin levels are shown in Table 1. Children exhibited the highest proportion of patent parasitaemia and relatively low levels of haemoglobin, which increased with age.

Table 2 shows the mean ELISA values for PRBC, NRBC, and the $M_r = 41\ 000$ and 31-1 polypeptides. There was some bias in the analysis of the results with the 1:100 dilution since 7%, 3%, and 18% of the values, respectively, were greater than 1.9 (saturation value) for the PRBC, $M_r = 41\ 000$, and 31-1 antigens. The results using the 1:2000 dilution are therefore also shown. At this dilution, only four sera still gave values greater than 1.9, but only on plates coated with 31-1 antigen. The mean ELISA values for PRBC, NRBC, and the $M_r = 41\ 000$ and 31-1 polypeptides of normal blood donors, patients with schistosomiasis, and patients with acute bacterial infection are shown in Table 3. Some of the patients suffering from schistosomiasis probably had had malaria, since four of them had antibody titres at dilutions of 1:80-1:160 (by indirect immunofluorescence). The results in

Table 1. Proportion of patients from the Gambia with patent parasitaemia and their haemoglobin levels

Age group (years)	No. of patients	Proportion with parasitaemia (%)	Mean haemoglobin level (g/dl)
1-2	27	96	8.5 \pm 1.3
3-5	63	95	10.1 \pm 1.7
6-9	76	91	11.6 \pm 1.5
10-14	71	80	12.0 \pm 1.4
15-24	109	47	12.5 \pm 1.7
25-44	140	24	12.6 \pm 2.0
45-75	81	21	12.1 \pm 2.0

Tables 2 and 3 indicate that the PRBC, $M_r = 41\ 000$ polypeptide, and 31-1 antigens can be used to detect antimalarial antibodies and that high antibody levels against NRBC are found frequently not only in patients with current or past malaria infections but also in those with infections unrelated to malaria. Regression analysis of the PRBC and NRBC data from the Gambian patients showed that the *r*-value was not significant at either 1:100 or 1:2000 dilutions, which suggests that a correction factor cannot be used to increase the sensitivity of the determination of levels of antimalarial antibody with PRBC as antigen. The regression coefficients for the other antigens investigated (Table 4) indicate that there is a good correlation between the values for PRBC and the $M_r = 41\ 000$ polypeptide, but only marginal correlation between PRBC and the 31-1 polypeptide or

Table 2. ELISA values (absorbance at $\lambda = 405$ nm) for patients from the Gambia

Antigen	Absorbance ($\lambda = 405$ nm)	
	Mean \pm S.D.	Range
Parasitized red blood cells	1.09 \pm 0.43 ^a (0.43 \pm 0.24) ^b	0.14-2.0 (0.03-1.2)
Normal red blood cells	0.33 \pm 0.17 (0.09 \pm 0.04)	0.06-1.3 (0.01-0.45)
$M_r = 41\ 000$ Polypeptide	0.75 \pm 0.34 (0.18 \pm 0.10)	0.04-2.0 (0.02-0.75)
31-1 Polypeptide	1.00 \pm 0.88 (0.15 \pm 0.26)	0.05-2.0 (0-2.0)

^d Titterskan, Flow Laboratories, Baar, Switzerland.

^e McGraw Hill Inc., New York, USA.

^a Figures refer to a serum dilution of 1:100.

^b Figures in parentheses refer to a serum dilution of 1:2000.

Table 3. ELISA values (absorbance at $\lambda = 405$ nm) for 50 control sera from normal blood donors, 10 patients with schistosomiasis, and 15 patients with acute bacterial infection

Antigen	Serum	Absorbance ($\lambda = 405$ nm)	
		Mean \pm S.D.	Range
Parasitized red blood cells	BD ^a	0.09 \pm 0.06	0.03–0.17
	Sch ^b	0.18 \pm 0.13	0.04–0.26
	ABI ^c	0.19 \pm 0.27	0.08–0.27
Normal red blood cells	BD	0.07 \pm 0.03	0.03–0.33
	Sch	0.18 \pm 0.04	0.09–0.54
	ABI	0.15 \pm 0.05	0.04–0.6
$M_r = 41$ 000 Polypeptide	BD	0.10 \pm 0.05	0.04–0.23
	Sch	0.22 \pm 0.11	0.13–0.55
	ABI	0.09 \pm 0.05	0.04–0.23
31–1 Polypeptide	BD	0.07 \pm 0.03	0.03–0.14
	Sch	0.23 \pm 0.10	0.13–0.55
	ABI	0.08 \pm 0.03	0.05–0.17

^a Blood donors.

^b Patients with schistosomiasis.

^c Patients with acute bacterial infection.

between the $M_r = 41$ 000 and 31–1 polypeptides. The distribution of sera that were positive for increasing ELISA values shown in Fig. 1 is regular for NRBC, PRBC, and the $M_r = 41$ 000 polypeptide but exhibits

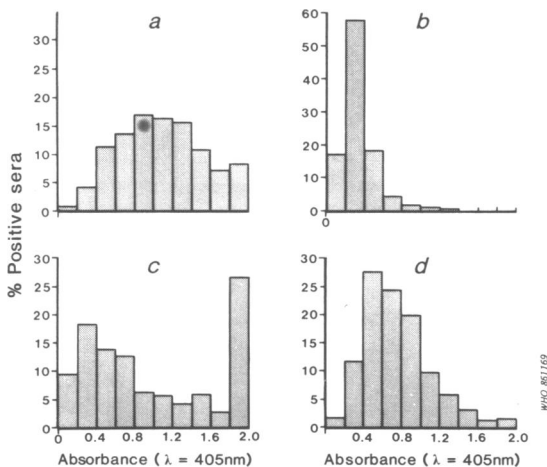


Fig. 1. Proportion of positive sera among the 567 samples from the Gambia plotted against the absorbance at $\lambda = 405$ nm for the four micro-ELISA assays. Serum dilution, 1:100 (*a* = parasitized red blood cells; *b* = normal red blood cells; *c* = 31–1 polypeptide; *d* = polypeptide of $M_r = 41$ 000).

greater variation for the 31–1 polypeptide.

One of the limitations of the micro-ELISA assay with PRBC as antigen is its relatively poor ability to detect antimalarial antibodies in young children (16). The results for the patients of various ages from the Gambia (Table 5) show that significant mean antibody levels against the four malaria antigen preparations described here occurred in young children and that levels of antibody increased with age. However, there was considerable variation between individuals, and this is reflected in the large standard deviations. Antibody levels against NRBC were relatively high and did not vary greatly for the different age groups; furthermore, there were no significant differences between the sexes in this respect. No comparison of the results for individuals with and without patent parasitaemia could be made since the majority of patients in the study exhibited parasitaemia; however, analysis of the results for the whole series of patients did not reveal a significant difference between these two groups (data not shown).

In order to evaluate the results obtained from the ELISA tests described here, baseline levels are needed, and for this purpose two reference values were selected for each malaria antigen preparation. For PRBC, these were the mean ELISA values for NRBC plus 2 or 3 standard deviations for patients with acute bacterial infection and an internal control defined as the mean value for NRBC plus 2 or 3 standard deviations for each age group. The values for patients with schistosomiasis were not used, since

Table 4. Correlation coefficients (*r*) between the ELISA results for the antimalarial antigens studied

Antigen	Antigen		
	Normal red blood cells	$M_r = 41$ 000 Polypeptide	31–1 Polypeptide
Parasitized red blood cells	$r = 0.05^a$ $P > 0.05$	$r = 0.57$ $P < 0.01$	$r = 0.21$ $0.01 < P < 0.05$
	$(r = 0.12)^b$ $(P > 0.05)$	$(r = 0.54)$ $(P < 0.01)$	(intercept not significant)
Normal red blood cells		$r = 0.32$ $P < 0.01$	$r = 0.25$ $P < 0.01$
		$(r = 0.28)$ $(P < 0.01)$	(slope not significant)
$M_r = 41$ 000 Polypeptide			$r = 0.22$ $0.01 < P < 0.05$
			(slope not significant)

^a Figures refer to a dilution of 1:100.

^b Figures in parentheses refer to a dilution of 1:2000.

Table 5. ELISA values (absorbance at $\lambda=405$ nm) for patients from the Gambia

Age group (years)	Antigen			
	Parasitized red blood cells	Normal red blood cells	$M_r=41\ 000$ Polypeptide	31-1 Polypeptide
1-2	0.67 ± 0.37 ^a	0.24 ± 0.14	0.47 ± 0.21	0.50 ± 0.55
3-5	0.86 ± 0.40	0.31 ± 0.19	0.60 ± 0.30	0.91 ± 0.70
6-9	1.04 ± 0.39	0.37 ± 0.21	0.70 ± 0.28	0.97 ± 0.64
10-14	1.10 ± 0.37	0.33 ± 0.16	0.79 ± 0.38	1.03 ± 0.70
15-24	1.07 ± 0.45	0.34 ± 0.13	0.75 ± 0.33	0.99 ± 0.66
25-44	1.22 ± 0.41	0.34 ± 0.15	0.81 ± 0.33	1.08 ± 0.70
45-75	1.25 ± 0.41	0.37 ± 0.19	0.90 ± 0.36	1.16 ± 0.66

^a Figures shown are the mean ± standard deviation.

some of them had antimalarial antibodies. For the $M_r=41\ 000$ and 31-1 antigens, the mean values for normal blood donors plus 2 or 3 standard deviations and those for patients with acute bacterial infection plus 2 or 3 standard deviations were selected as the normal upper limits. The proportion of patients with ELISA values greater than the mean control values plus 2 or 3 standard deviations are shown in Table 6. As expected, the proportion of such patients increased with age for any of the malaria antigen preparations used. These results also indicate that the

presence of anti-NRBC antibodies may result in false positive readings with PRBC. Also, the PRBC micro-ELISA assay did not detect antimalarial antibodies reliably in children less than 6 years of age; however, use of either the $M_r=41\ 000$ or 31-1 antigen gave satisfactory results, even in young children.

To assess the specificity of the micro-ELISA tests described here, two sera from patients from Côte d'Ivoire were incubated with various concentrations of each of the four antigens investigated and tested on micro-ELISA plates coated with NRBC or malaria

Table 6. Proportion of patients from the Gambia with ELISA values for parasitized red blood cells, $M_r=41\ 000$ polypeptide, and 31-1 polypeptide greater than the reference mean plus 2 or 3 standard deviations

Age group (years)	Proportion of patients (%)											
	Parasitized red blood cells				$M_r=41\ 000$ Polypeptide				31-1 Polypeptide			
	Acute bacterial infection		Normal red blood cells		Normal blood donors		Acute bacterial infection		Normal blood donors		Acute bacterial infection	
	+2SD ^a	+3SD	+2SD	+3SD	+2SD	+3SD	+2SD	+3SD	+2SD	+3SD	+2SD	+3SD
1-2	30	19	56	33	93	86	93	89	82	78	78	74
3-5	56	37	62	43	98	92	98	95	97	95	97	91
6-9	78	57	74	57	99	97	100	97	97	96	96	90
10-14	86	59	87	78	100	100	100	100	95	93	93	92
15-24	73	52	86	76	99	99	99	98	100	98	98	97
25-44	88	64	93	84	99	99	100	98	100	96	96	94
45-75	88	69	86	72	100	100	100	100	99	98	99	95

^a SD = standard deviation.

Table 7. Specificity of the micro-ELISA assays described

Antigen	% Binding inhibition:											
	Parasitized red blood cells			Normal red blood cells			$M_r = 41\ 000$ Polypeptide			31-1 Polypeptide		
	1 × ^a	10 ×	100 ×	1 ×	10 ×	100 ×	1 ×	10 ×	100 ×	1 ×	10 ×	100 ×
Parasitized red blood cells	5	30	67	4	19	44	—	—	<10	—	—	<10
	7	37	83	3	22	45	—	—	10	—	—	<10
Normal red blood cells	4	18	37	15	50	79	—	—	<10	—	—	<10
	5	20	40	13	47	82	—	—	<10	—	—	<10
$M_r = 41\ 000$ Polypeptide	—	—	<15	—	—	<15	10	53	87	—	—	<15
	—	—	<10	—	—	<10	7	55	78	—	—	<15
31-1 Polypeptide	—	—	<10	—	—	<10	—	—	<10	15	66	93
	—	—	<15	—	—	<10	—	—	<10	13	70	89

^a Relative concentrations of antigen.

antigens. The results are shown in Table 7. Each of the antigenic preparations almost completely inhibited the binding of antimalarial antibodies to plates coated with the homologous antigen. There was no significant inhibition with heterologous antigenic preparations except for the NRBC antigen, which significantly decreased the ELISA values for plates coated with PRBC.

DISCUSSION

The results we have described, which are consistent with those reported previously in a study of individuals from the Gambia (15), indicate that there is a clear relationship between a patient's age, the presence of patent parasitaemia, as well as the levels of haemoglobin and antimalarial antibodies. In addition, our results confirm that individuals who live in areas where malaria is endemic have high levels of antibody against NRBC (10, 11). In contrast, there was no direct correlation between levels of antibodies against PRBC and those against NRBC; also high levels of antibodies against NRBC were frequently detected in patients with infections unrelated to malaria, e.g., acute bacterial infections. This suggests that the ELISA method with PRBC antigens cannot be used for the specific and quantitative determination of antimalarial antibodies, and this has frequently been underestimated in seroepidemiological studies that have used solid-phase assays and PRBC extracts to detect antimalarial antibodies (9, 11, 16). The relative inefficiency of PRBC as a source of antigen becomes apparent when mean levels of antibody against NRBC are included as controls, since the PRBC micro-ELISA test does not reliably determine antimalarial antibodies in children. An

additional limitation of PRBC as a source of antigens is that its preparation cannot be standardized.

In order to overcome these problems, we developed two micro-ELISA assays that use purified parasite components as sources of antigens. For the first of these antigens, we selected a parasite component of $M_r = 41\ 000$ present in the rhoptries of *P. falciparum* merozoites (12) and for the second, a fusion polypeptide of $M_r = 23\ 000$, obtained by DNA recombinant techniques, which contains at least some of the epitopes exposed in the major surface component of *P. falciparum* merozoites (13, 17). Using these polypeptides, we were able to standardize the assays by determining the concentration of polypeptides required for optimum coating of the micro-ELISA plates. Another advantage of these assays is the low background measured using sera from individuals never exposed to malaria and sera from individuals suffering from infections unrelated to malaria. By utilization of sera from individuals living in malaria endemic areas, it is shown that these tests can detect malaria antibodies in the majority of young children living in areas where malaria is endemic and that, as expected, antibody levels against the malaria polypeptides increase with age. A possible difficulty in using defined antigens may be the presence of immunodominant epitopes, such as repeating sequences of amino acids, that may not be represented in all isolates of *P. falciparum* (13, 18). This seems not to be the case for the $M_r = 41\ 000$ polypeptide, since repartition of the ELISA values is symmetric and similar for both this and the PRBC antigens; furthermore, 10 monoclonal antibodies against the $M_r = 41\ 000$ antigen reacted with 10 different isolates of *P. falciparum*. In contrast, the polypeptide of $M_r = 190\ 000$ –200 000 from which the

31-1 polypeptide is derived is antigenically diverse in different strains of *P. falciparum* (19). The 31-1 fusion polypeptide contains a region with several repeated amino acid sequences (Ser-Gly-Gly-Ser-Val-Ala) flanked by regions having a unique sequence (13). Using synthetic peptides corresponding to these repeat or flanking sequences, we found that more than 60% of the antibodies present in highly positive sera were directed against the repeat portion of the fusion polypeptide (Perrin et al., unpublished data). Similarly, a cDNA clone coding for part of the polypeptide of $M_r = 190\ 000$ – $200\ 000$ obtained from a *P. falciparum* isolate from Papua New Guinea had similar amino acid sequences for the flanking regions but also a repeat portion with a different amino acid sequence (R. Coppel, personal communication). These observations may explain the relatively low levels of antibody found in some individuals when the 31-1 antigen was used. To improve the micro-ELISA assay using this latter antigen, we are currently attempting to excise the repeat portion of a cDNA clone that corresponds to the 31-1 polypeptide but codes for a longer fragment of the $M_r = 190\ 000$ – $200\ 000$ polypeptide. The resultant

fusion polypeptide will therefore contain a large number of the epitopes present in the native molecule, thereby increasing the sensitivity of the assay; and will be devoid of epitopes involved in antigenic diversity, hence enhancing its specificity.

The practical use of purified polypeptides as a source of antigens necessitates the availability of methods for their large-scale production. This now presents no difficulties for the 31-1 polypeptide, which can be prepared 90% pure in 20–30 mg amounts from 3 litres of *E. coli* cultures containing the plasmid that expresses this antigen.

The $M_r = 41\ 000$ and $200\ 000$ polypeptides, from which the 31-1 polypeptide was derived, have been shown to induce a protective immune response in monkeys (12, 20). However, the results presented here do not indicate, on an epidemiological basis, whether or not antibodies against these polypeptides provide an index of protection. Although antibody levels against these polypeptides increase with age, and adults are more resistant to malaria, the wide range of results within each age group does not permit any conclusion regarding the protective nature of these antibodies.

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RÉSUMÉ

ANTIGÈNES DE *PLASMODIUM FALCIPARUM* POUR LA SÉROLOGIE DU PALUDISME

La mise au point de tests diagnostiques sensibles, spécifiques et économiques pour la mesure des anticorps antipaludiques revêt une grande importance. De tels tests sont nécessaires à la conduite d'études épidémiologiques qui permettent une évaluation précise de l'impact des campagnes de la lutte contre le paludisme. À l'avenir, ces tests auront également une grande utilité pour mesurer l'efficacité des essais de vaccination contre le paludisme. Un des obstacles majeurs à la mise au point de tests immunodiagnostiques réside dans l'absence de standardisation des tests; ceci est principalement dû à un manque d'antigènes bien caractérisés. Dans la présente étude, nous avons employé 3 préparations d'antigènes de *P. falciparum* pour mettre au point trois tests différents, en employant une méthode immuno-enzymatique de type ELISA. Les 3 antigènes employés pour former la phase solide liée à des cupules de polystyrène étaient:

- un extrait de cultures de globules rouges infectés par *P. falciparum*
- un polypeptide de $M_r = 41\ 000$ présent dans les rhoptries de *P. falciparum*. Ce polypeptide a été purifié par chromatographie d'affinité
- un polypeptide (31-1) produit par génie génétique, qui correspond à un fragment d'un antigène de surface des schizontes et mérozoïtes de *P. falciparum*.

Nous avons de plus utilisé comme témoin une quatrième solution antigénique: un extrait de globules rouges normaux. Dans la procédure expérimentale suivie, les cupules de polystyrène formant des microplaques ont été incubées avec la concentration optimale de chacun des antigènes, puis lavées et l'on a ensuite ajouté des solutions des sérums humains à tester. La fixation des anticorps sur les cupules enduites d'antigènes est ensuite mise en évidence par

un conjugué phosphatase alcaline — immunoglobuline anti-Ig humaine.

Les sérums de 567 habitants d'une zone d'endémie palustre en Gambie ont été utilisés pour évaluer la spécificité et la sensibilité des différents tests micro-ELISA. Il est apparu que les 3 tests micro-ELISA utilisant sur leur phase solide des antigènes du paludisme permettraient de détecter les anticorps antipaludiques chez les adultes dans plus de 98% des cas pour les tests utilisant l'antigène de $M_r = 41\ 000$ et le polypeptide 31-1, et dans 70 à 85% des cas, en employant l'extrait de globules rouges infectés par *P. falciparum*. En utilisant ce dernier extrait, il n'a pas été possible de mettre en évidence d'une façon sensible, sûre et reproductible, les anticorps antipaludiques chez les jeunes enfants, alors que les deux autres tests donnaient des résultats satisfaisants. Le manque de sensibilité et de spécificité du

test utilisant l'extrait antigénique de culture de *P. falciparum* est dû d'une part à la présence d'antigènes de globules rouges dans l'extrait antigénique utilisé, et d'autre part à la présence fréquente d'anticorps anti-globules rouges dans les sérums des habitants des régions d'endémie palustre.

L'utilisation des antigènes de $M_r = 41\ 000$ et 31-1 permet une standardisation des tests visant à la détection des anticorps antipaludiques, permet de détecter ces anticorps avec une fréquence accrue par rapport au test basé sur l'emploi de globules rouges infectés, et s'accompagne d'une grande spécificité. Les problèmes liés à la production de ces antigènes et à leur emploi pour l'évaluation des réponses spécifiques, après vaccination par exemple, sont discutés en détail.

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