JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1991, p. 510–518
0095-1137/91/030510-09$02.00/0

Vol. 29, No. 3

Development of Natural Immunity in *Plasmodium falciparum* Malaria: Study of Antibody Response by Western Immunoblotting

JACQUES THELU,[1]* ILMASSE SHEICK-ZAKIUDDIN,[1] CHRISTIAN BOUDIN,[1,2] FRANÇOIS PEYRON,[1] STÉPHANE PICOT,[1] AND PIERRE AMBROISE-THOMAS[1]

Département de Parasitologie, Mycologie Médicale et Moléculaire, Centre National de la Recherche Scientifique URA 1344, Faculté de Médecine, Université Joseph Fourier Grenoble 1, 38706 La Tronche, France,[1] and ORSTOM, Centre Muraz, Bobo-Dioulasso, Burkina Faso[2]

Received 27 June 1990/Accepted 7 December 1990

A longitudinal study was carried out in Burkina Faso to investigate the natural development of the immune response to *Plasmodium falciparum* malaria. Three bleedings were carried out before, during, and after the seasonal peak of transmission. Detailed antigen mapping and antibody prevalence of the 248 collected serum samples were established by immunoblotting on the basis of several epidemiological and biological parameters. An improved Western immunoblotting system was used to analyze up to 67 serum samples on each nitrocellulose sheet. This system allowed us to perform the entire study with strictly comparable conditions. Two different blood-stage antigens (exoantigens and somatic antigens) were used to analyze the distribution of different classes and subclasses of immunoglobulins according to the age of the individuals, the presence or absence of a malarial attack, the transmission period, the origin of parasite isolates, and the response to intraerythrocytic stages. Although this analysis emphasizes strong individual variations, reactions with two major antigens of 115 and 103 kDa were especially noted. These antigens induced high antibody levels and prevalences but were probably not involved in protection. The prevalence of immunoglobulin G (IgG) antibodies differed by isotype. Most of antigens stimulating IgG production were also responsible for the IgM antibody response. The role played by these antibodies in the development of natural immunity against malaria is discussed.

In endemic areas, young children and newly arrived people are particularly vulnerable to malaria. Thereafter, following numerous infective mosquito bites, a functional but nonsterilizing immunity gradually develops. The identification of the antibodies involved in this natural protection is necessary not only to assess the feasibility of a vaccine but also to control any mass antimalarial action which, in turn, should not elicit any decrease in the vital immunological barrier.

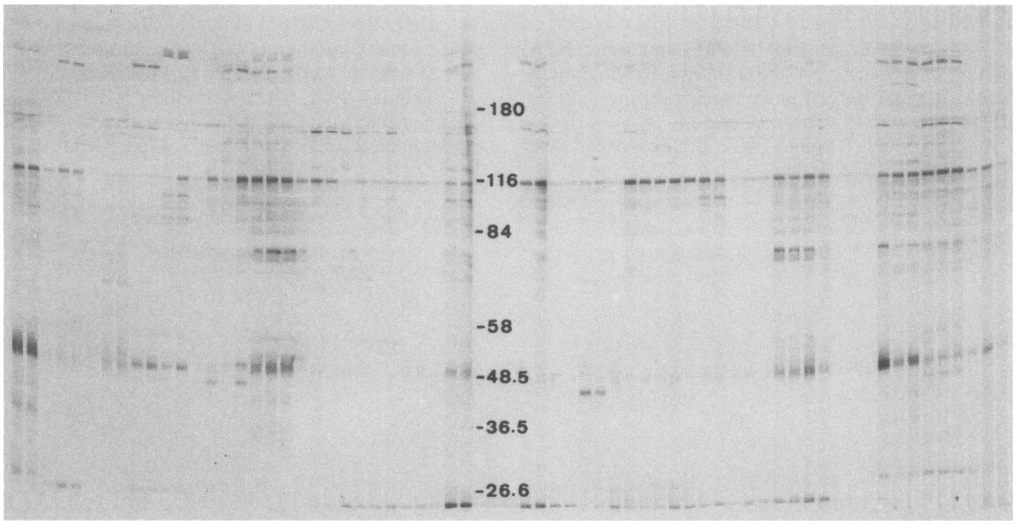

FIG. 1. IgG antibodies detected by Western blotting with the apparatus described in the text. Somatic antigens from strain SGE_1 cross-reacted with diluted sera. Duplicates or triplicates correspond to bleedings done at different periods. Numbers are masses in kilodaltons.

Many malarial antigenic determinants were found to induce an antibody response, but their use as vaccines was insufficient to prevent a severe malarial attack (4, 14, 33). These studies emphasized the fact that complex defense mechanisms require cooperation between antibodies and a cellular immune response to limit parasite proliferation (15,

* Corresponding author.

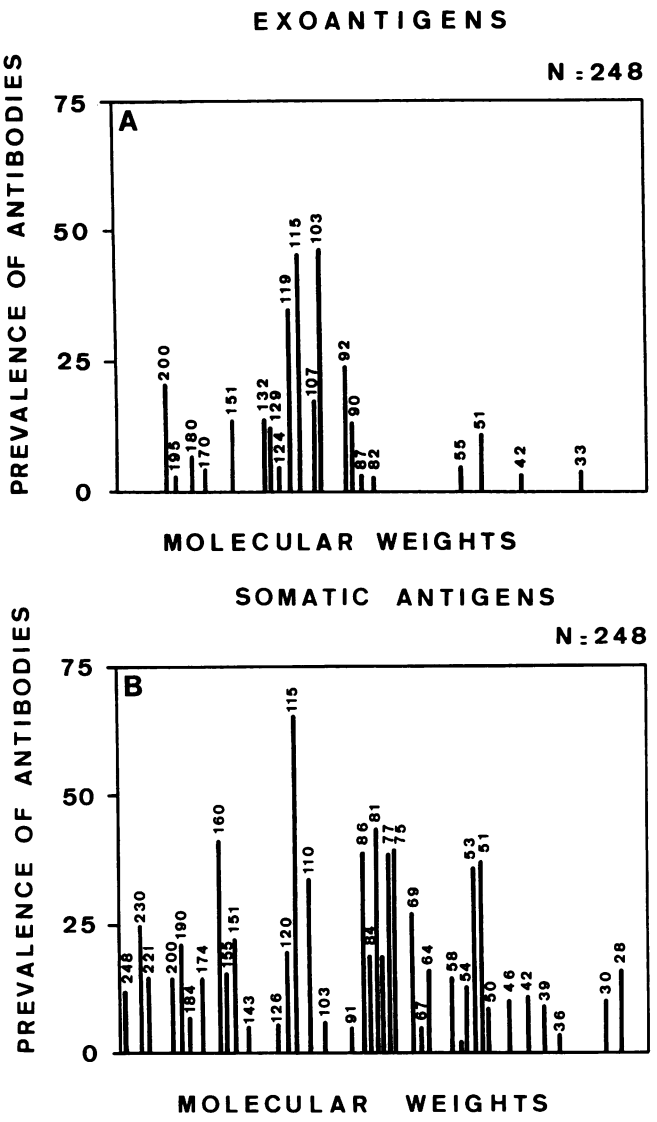

FIG. 2. Distribution of antimalarial IgG antibodies detected by Western blotting in 248 serum samples taken from people living in an area in which seasonal malaria was endemic. Each bar represents the percentage of sera containing antibodies related to each *P. falciparum* parasite-specific antigen. The numbers indicate the molecular weights (10^3) of the related antigens from two erythrocytic-stage extracts: exoantigens from serum-free supernatant (A) and crude somatic antigens (B), both prepared from *P. falciparum* SGE_1.

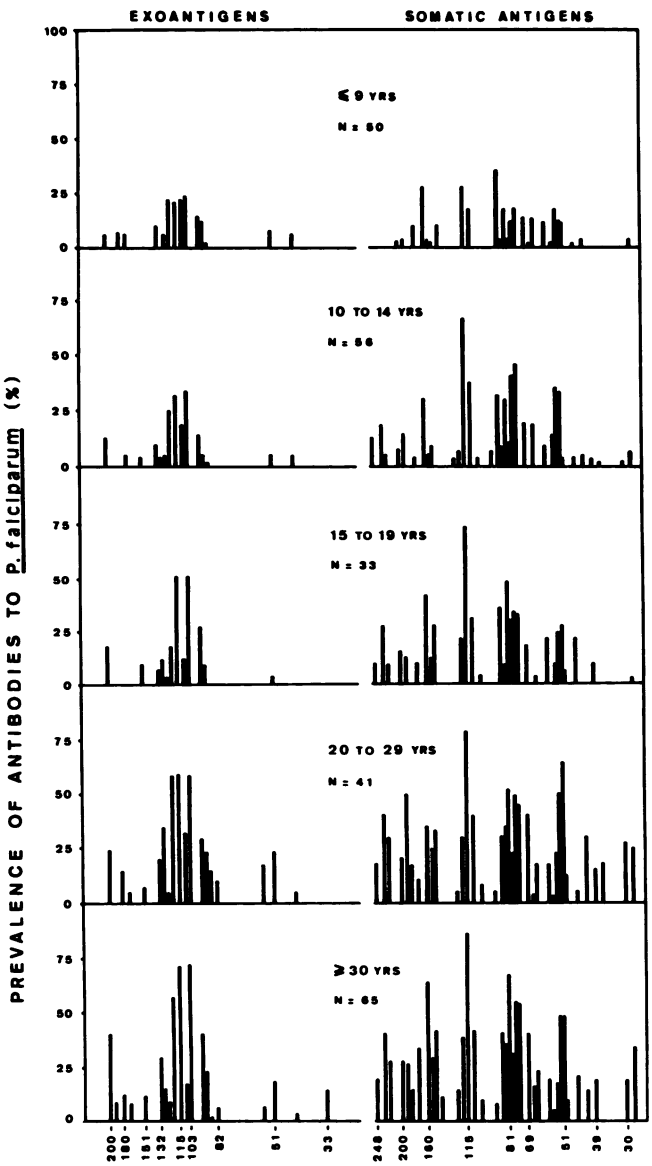

FIG. 3. Distribution of antibodies to *P. falciparum* by age. The studied population was divided in five different groups, according to the ages of the individuals. As in Fig. 2, antigens were taken from an asynchronous culture (SGE_1). The general prevalence of IgG-positive responses increased with age. For individuals over 20 years old, the distribution of the antibody responses against *P. falciparum* was quite stable. Numbers at the bottom are molecular weights (10^3) of antigens.

34, 42, 45). Naturally acquired humoral immunity must be evaluated with sera collected from people living in endemic areas, and it is for this reason that we investigated the prevalence of antibodies by Western immunoblotting, a technique that yields much information about antigens. This work was conducted in a well-studied area near Bobo-Dioulasso in Burkina Faso (7, 13, 35, 36).

MATERIALS AND METHODS

Study area and population. The investigation was carried out in a Sudanese savanna area in Burkina Faso. Two villages in which seasonal malaria was endemic were chosen. Parasitological and clinical studies were correlated to a cycle of malarial transmission from June 1987 to January 1988. Maximal malaria incidence occurs during the rainy season, from May to October, and decreases during the 6 months of the dry season. The population is primarily exposed to *Plasmodium falciparum*, with rare infections caused by *P. malariae* and *P. ovale*. Eighty-five subjects from 4 to 67 years old were examined twice a week by a physician, and chloroquine was administered in cases of malarial attack. The disease was confirmed when febrile episodes and infected cells (10,000 parasitized erythrocytes [RBC] per mm^3) were observed and when patients recovered following chloroquine treatment.

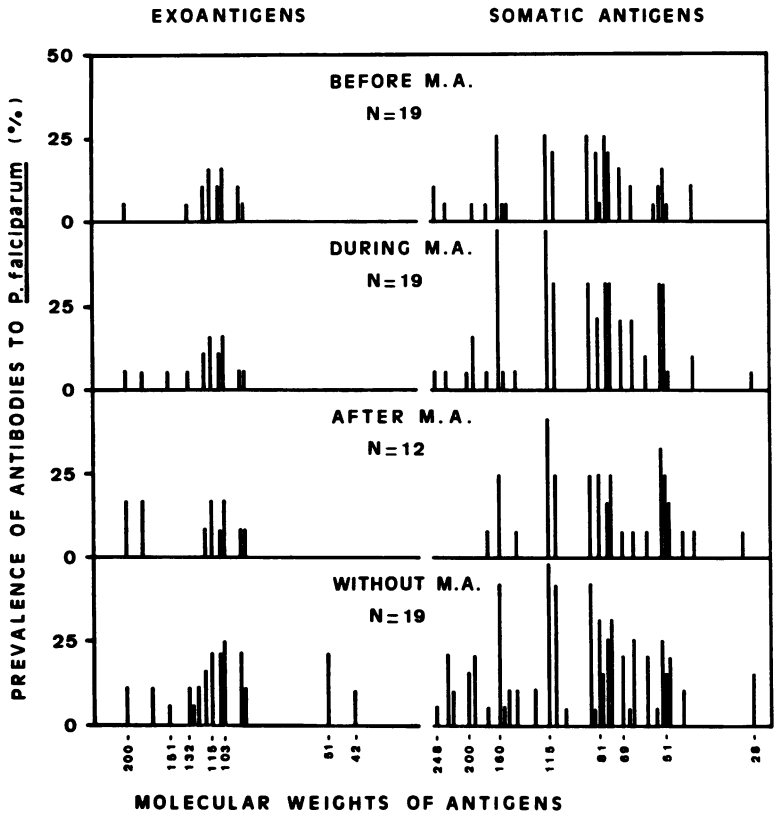

FIG. 4. Prevalence of antibodies against the asexual blood-stage antigens in malaria-positive and malaria-negative children. The latter group consisted of the same number of healthy children of the same age (up to 15 years old) as in the former group and were randomly chosen. The prevalence bar graph for the rainy season was used as a reference (not shown). Molecular weights are in thousands. M. A., Malarial attack.

Serum. Volunteers in several families were bled at three different periods: June, at the beginning of the transmission period; August and September, during the peak of transmission; and January, at the end of the transmission period. The 248 samples were frozen in liquid nitrogen and stored at −70°C.

Twenty-four nonimmune serum samples from European individuals who had never been in contact with malaria were tested as controls.

Parasite strains and cultures. In most cases, a *P. falciparum* strain originating from Gambia was used (SGE_1). It has been maintained in an in vitro culture for 12 years. In one experiment, four other strains were cultivated: an isolate from Banghi in the Republic of Central Africa (BNG), an isolate from the Ivory Coast Republic (JN8), and two cloned strains, Honduras and Indochina. In vitro culturing was done on A^+ RBC with a 10% hematocrit, RPMI 1640 (GIBCO) medium, and 10% human serum (A^+ individuals) in a 5% CO_2 atmosphere at 37°C, with daily medium changes (41). To obtain serum-free medium, we washed parasitized RBC twice with RPMI 1640 and left them in culture conditions, without human serum, for 48 h. For the mass production of parasites, the previously described automated cell culture unit was used (38), and the culture was synchronized by sorbitol treatment (23).

Antigens. The exoantigens were obtained from culture supernatants free of human serum, concentrated 10 times by ultrafiltration (Amicon), dissolved in 0.4 M Tris buffer containing 1% SDS, 1% 2-mercaptoethanol, 13% glycerol, and 1% bromophenol blue, and boiled for 1 min.

To prepare somatic antigens, we harvested parasitized RBC, washed them three times in isotonic phosphate buffer (PBS; 50 mM, pH 7.2), and lysed them with saponin to give a final concentration of 0.4 g/liter. Parasites were washed four times in PBS (10,000 × *g* for 10 min), and the pellet was resuspended in Tris buffer (described above). The final protein concentration was adjusted to 8 mg/ml with a detergent-compatible protein assay (Pierce). Control antigens were prepared in the same manner with an equal number of RBC without parasites. To obtain merozoites, we treated RBC with trypsin to prevent reinvasion (12). Fresh RBC were incubated for 1 h at 37°C in RPMI 1640 containing 1 mg of bovine trypsin (Merck) per ml and washed twice with RPMI 1640 (400 × *g* for 10 min) before cultivation. Antigens were immediately electrophoresed or stored at −70°C as necessary.

Immunoblots. To perform immunoblots in strictly comparable conditions, we used sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (22) of antigens on eight simultaneously molded gel slabs (220 by 220 by 1 mm). Separation was done with a 7 to 10% polyacrylamide gel gradient containing 0.27% *N,N'*-methylenebisacrylamide and 0.1% sodium dodecyl sulfate in Tris hydrochloride buffer (0.37 M, pH 8.8). The stacking zone contained 5% acrylamide, 0.13% *N, N'*-methylenebisacrylamide, and 0.1% sodium dodecyl sulfate in Tris hydrochloride buffer (0.12 M,

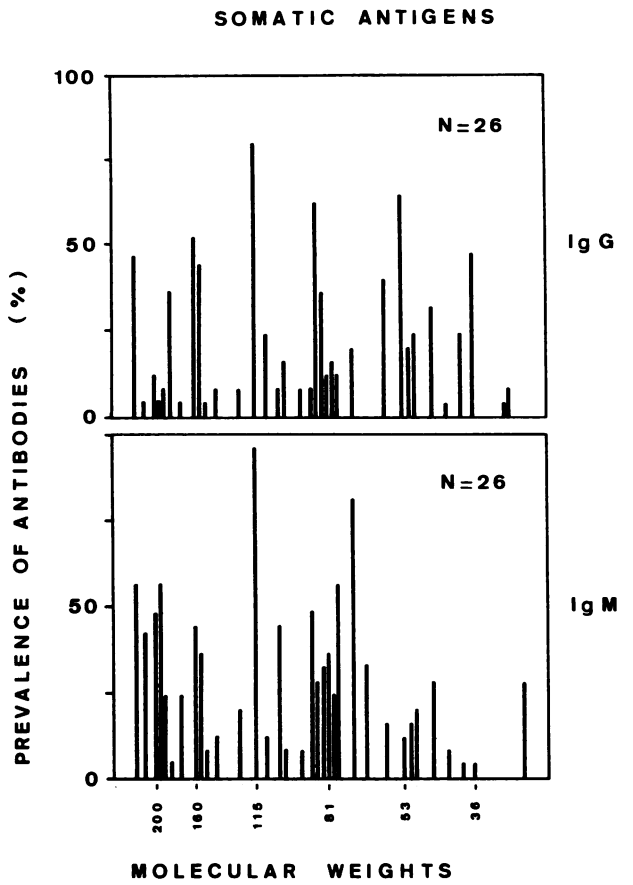

FIG. 5. Prevalence of anti-*P. falciparum* IgM antibodies in 26 selected serum samples recognizing several antigens in the blots shown in Fig. 2. The prevalence differed from that of IgG antibodies in the studied area. Nevertheless, IgM and IgG seemed to recognize the same target antigens, as shown by their similar molecular weights (10^3), in both crude extracts.

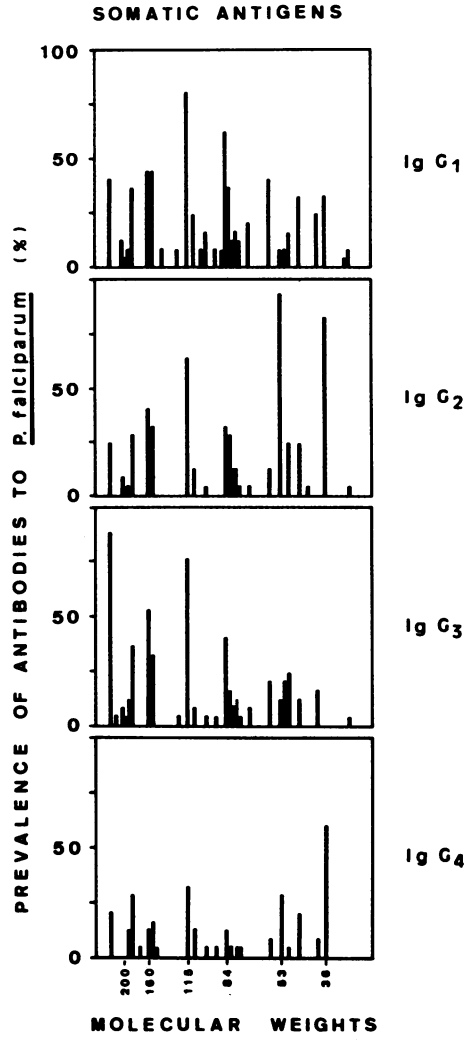

FIG. 6. Distribution of IgG subclasses. The same selected positive sera as those used in Fig. 5 were used to determine the distribution of IgG subclasses. Each studied isotype provided very different patterns of antibody reactivity. Antibodies of each isotype were directed against the same set of antigens, but their quantity or affinity differed. Molecular weights are in thousands.

pH 6.8). The antigen to be fractionated (0.3 ml) was loaded onto the top of a gel slab in a single 200-mm-wide well and electrophoresed at 180 V for 4 h. Twenty-one molecular weight markers from three different kits were used (Pharmacia, Sigma, and GIBCO-Bethesda Research Laboratories). When needed, gels were stained with silver nitrate (28) or with Coomassie blue. Macromolecules were simultaneously transferred onto nitrocellulose sheets (Amersham; 0.45 μm) soaked in 20 mM Tris-glycine buffer (pH 8.3)–20% methanol. Each gel slab was separated from the next nitrocellulose sheet with four filter papers (Whatman no. 1) soaked in the same buffer. This assemblage was sandwiched between two graphite slabs (250 by 250 by 20 mm) acting as electrodes, and electrotransfer was done at 30 V for 2 h. Thereafter, nitrocellulose sheets were saturated for at least 10 min in PBS–5% nonfat milk and processed in a press. This previously described system (39) was improved to allow the analysis of 67 serum samples on each sheet. This homemade apparatus consisted of two blocks of plastic (250 by 150 by 20 mm), between which were placed a flat rubber gasket of the same size and a nitrocellulose sheet on which electrotransfer had already taken place. The blot was pressed firmly by 10 screws set all around the system. On the inside of the upper block, parallel troughs (100 by 2 by 2 mm) were drilled at intervals of 1 mm, creating 67 lanes on the nitrocellulose sheet that were perpendicular to antigenic bands. Diluted sera (1/30 in PBS–5% nonfat milk) introduced into each longitudinal trough through upright holes pierced through the upper block were reacted with antigens for 1 h at 37°C. After washing was done (three times for 5 min each time in PBS–0.05% Tween 20), antibodies were revealed simultaneously in strictly comparable conditions without the strips of nitrocellulose having to be separated (Fig. 1). The protocol used the following peroxidase-labeled antihuman immunoglobulins furnished by NORDIC laboratories: total immunoglobulin G (IgG), IgM, IgE, and IgA, from goat, diluted to 1/500 and IgG1, IgG2, IgG3, and IgG4, from sheep, diluted to 1/200. The immunoglobulins were diluted in PBS–5% nonfat milk and incubated for 1 h at 37°C. The specificities of the antisera were determined by the manufacturer in double immunodiffusion and in immunoelectrophoresis against isolated and purified myeloma proteins of all known subclasses. Sheets were washed and developed with 1 mg of 4-chloro-1-naphthol (Sigma) per ml–20% methanol in PBS plus 1 μl of

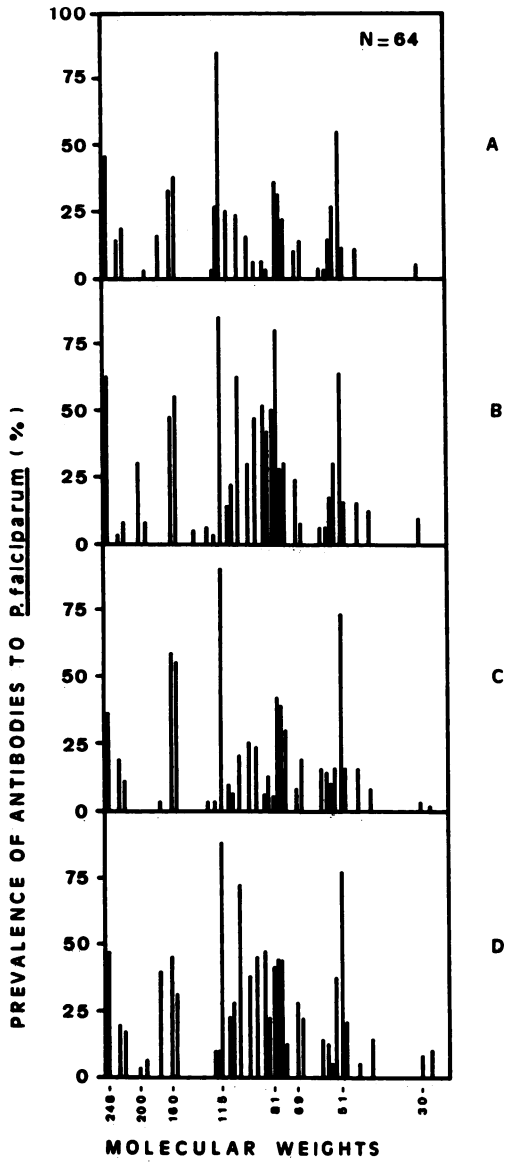

FIG. 7. Prevalence of antibodies against various *P. falciparum* strains. A batch of 64 randomly chosen serum samples was used to determine the prevalence of antibodies directed against four *P. falciparum* strains: BNG (A), JN8 (B), Honduras (C), and Indochina (D). Molecular weights are in thousands.

hydrogen peroxide per ml (110 volumes). A color intensity level coefficient ranging from 1 to 3 was assigned to each band. The results were analyzed with a data Base III computer program.

RESULTS

The IgG antibody patterns obtained with all of the sera are summarized in Fig. 2A and B. The numbers of antigenic macromolecules assessed and additional processed products were greater for detergent-solubilized parasites (38 molecules) than for supernatant exoantigens (20 molecules). In fact, in both extracts, many antigens had similar or only slightly different molecular weights and therefore could have been identical. It was also observed that antibodies against somatic sodium dodecyl sulfate-soluble products predominated (Fig. 2B) over those against exoantigens (Fig. 2A).

No band was revealed with the 24 nonimmune serum samples from European individuals who had never been in contact with malaria (data not shown). Nevertheless, when immune African sera were incubated with the blotted supernatant from nonparasitized RBC, one band was revealed (data not shown). This unique, nonspecific reaction with a low-molecular-weight molecule that migrated with the front was discarded from all data. No reactivity was detected with the somatic counterpart from noninfected RBC.

The proportion of responders to *P. falciparum* increased very significantly with age, until a steady state was reached for individuals about 20 years old (Fig. 3). As with the prevalence of antibodies, the reacting-antigen subset increased as children got older.

During this survey, 19 children less than 15 years old had clinically detectable malarial attacks that responded to chloroquine treatment. For these patients, the incidence of the disease was assessed by comparing the spectra before, during, and after the febrile episode (Fig. 4). They presented the same general structure, but several antibody frequencies increased rapidly when infection appeared (i.e., 190-, 160-, 115-, 110-, 86-, 75-, 64-, and 81-kDa somatic antigens) and other, additional antibodies were produced (i.e., 180- and 151-kDa exoantigens and 200-, 143-, and 28-kDa somatic antigens). About 5 months later, frequencies fell back to the original position (i.e., antibodies to 160-, 110-, 86-, 75-, 64-, and 81-kDa somatic antigens) or even disappeared (i.e., 151-kDa exoantigens and 200-kDa somatic antigens). The antibody titers estimated by the color intensities of the bands reflected the same increase. This boost in the frequency of malaria antibodies was observed for the entire adult population just after the beginning of the transmission season (data not shown). Furthermore, there is some evidence to suggest higher numbers of antibodies in individuals who have never had a malarial attack.

Twenty-six serum samples recognizing several antigens in the blots were selected to compare IgG, IgM, and IgG isotype responses. Many antigenic determinants from the parasite were active for both IgG and IgM (Fig. 5). The production of IgM was mainly stimulated by the heavier macromolecules. The four IgG subclasses were tested against intraerythrocytic-stage parasites (Fig. 6). A few somatic antigen determinants (4 of 30) were strongly active (the prevalence was up to 80%) for specific isotype expression, whereas the other antigens weakly stimulated isotype expression (the prevalence was about 20%). Neither IgE nor IgA antibodies were found to react with parasite antigens.

Immunoblotting conducted with 64 serum samples from patients with strong somatic antigen responses to the four isolates from different countries resulted in characteristic patterns (Fig. 7). Only a few antigenic molecules (160, 115, 81, and 51 kDa) capable of inducing strong responses were found in all of the isolates, as shown for strain SGE_1 (Fig. 2A and B). However, some antigens (200, 174, 103, 91, 84, and 28 kDa) were involved in isolate-dependent immunoblotting reactions. Nevertheless, despite the different sources of the isolates, the spectra had the same general aspect.

Time-course experiments revealed a stage-dependent enhancement of IgG (Fig. 8) and IgM (Fig. 9) prevalence, from early trophozoites to free merozoites. The number of active macromolecules increased progressively, but the prevalence of reciprocal antibodies did not increase in a linear manner. Soluble antigens in the medium did not follow the same stage evolution. Metabolic extracts from ring-form parasites did

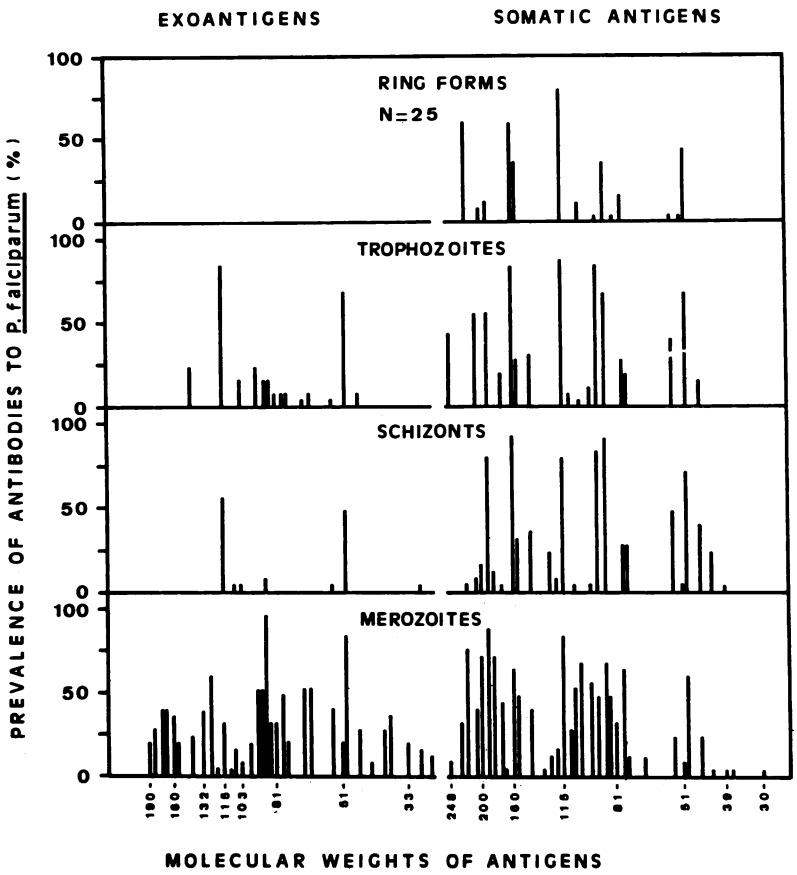

FIG. 8. Identification of stage-specific antigens. Stage-specific antigens were identified with synchronized cultures and 25 highly responsive serum samples. IgG prevalences indicate that crude extracts of parasites of each developmental stage contained many detectable antigens. Molecular weights are in thousands.

not contain any detectable antigens, and only a few antigens were present in the schizont medium. Because of the lysis of infected RBC, antigen release into the merozoite medium was very consistent.

DISCUSSION

Western blotting with both the parasite lysate and the crude culture supernatant of *P. falciparum* is a powerful epidemiological tool, since it allows the precise measurement of antibody patterns. Few antigenic determinants were strongly reactive in terms of high antibody frequencies and levels: the prevalence of the 115-kDa antigen was up to 60% (Fig. 2A and B). This prevalence was reached by the time children were about 10 years old (Fig. 3) and then remained constant. IgG isotypes 1, 2, and 3, specific for the 115-kDa antigen, were found to have the same intensity (Fig. 6). This antigen was also strongly recognized by IgM (Fig. 5 and 9) and was not strain specific (Fig. 7). About 50% of the children having undergone a malarial attack had anti-115-kDa antigen antibodies (Fig. 4) at a level similar to that in the control children (no malarial attack). Therefore, these antibodies alone were not sufficient and may not even be involved in malaria protection. A major 115-kDa parasite polypeptide was present in the crude supernatant and in the somatic antigen. The concordance of cohorts that produced anti-115-kDa antigen antibodies against both antigens led us to believe that the same molecule was probably present in both cellular extracts and supernatants. It was expressed very early in the parasite cycle and seemed to be maximal as soon as ring-form parasites occurred (Fig. 8). A considerable amount of 115-kDa antigen was released into the medium as soon as trophozoites became mature. Other authors reported that a protein with the same M_r was highly immunoreactive (26, 30, 31, 46).

A strongly reactive 103-kDa antigen was present in both crude cellular extracts (Fig. 2B). As sera with anti-103-kDa somatic antigen antibodies also reacted with a 103-kDa exoantigen, one can suggest that these antigens are identical and may even be very actively excreted.

Other major immunoreactive components were found in somatic extracts, i.e., 160, 110, 86, 81, 77, 53, and 51 kDa (Fig. 2B), but their corresponding prevalences were similar in both groups (children with and without malarial attack) (Fig. 4). Nevertheless, many determinants showing a low reactivity (170-, 129-, 124-, and 42-kDa exoantigens and 221-, 126-, 84-, 67-, and 46-kDa somatic antigens) were only present in the control children. Many antigens having these masses have already been described as playing a putative protective role: the 220-kDa glycosylated antigen (20, 37), the 126-kDa antigen from the parasitophorous vacuole (1, 8, 9), at least two 45-kDa antigens (24, 29), and other antigens of 42 and 83 kDa (merozoite surface fragment of the 195-kDa precursor) that conferred incomplete protection in *Aotus trivirgatus* (16–18, 32).

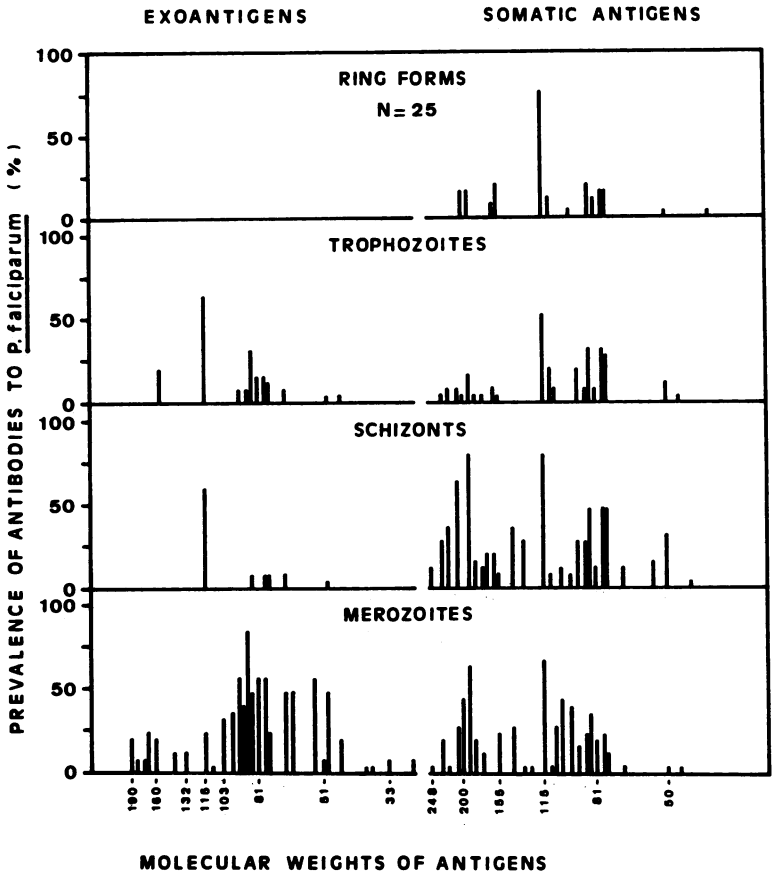

FIG. 9. Western blotting assessment of IgM antibodies specific for each culture stage of *P. falciparum*. Results were similar to those for IgG (Fig. 8). Molecular weights are in thousands.

The 155-kDa parasite component revealed by blotting should correspond to the Pf 155 or ring-infected erythrocyte surface antigen (2, 43). Our study allowed us to confirm that a 155-kDa antigen is present in the early intraerythrocytic stages (ring forms) of wild-type isolates and cloned strains and increases up to the merozoite stage; hence, the anti-155-kDa antigen antibody increases with age. Surprisingly, the 155-kDa antigen was only weakly detected in supernatants during merozoite release (Fig. 8). This result may have been due to dilution in the culture medium or to the processing of this molecule.

The immunoblotting patterns indicated that immune adult sera contain antibodies of all IgG subclasses specific for the whole series of somatic antigens, as previously observed for ring-infected erythrocyte surface antigen (44). IgG1 was predominant against malarial antigens, and IgG4 was the least prevalent subclass (Fig. 6). It is noteworthy that in this kind of assay there might be an excess of antibodies for certain antigens. Therefore, competition for binding to certain epitopes should occur between the different antibody isotypes, and the observed results may not represent the true isotype pattern.

The detailed IgM mapping (Fig. 5 and 9) revealed the high degree of complexity of the stimulating antigen set and the high levels, which may have been a consequence of constant antigenic stimulation due to recurrent bites by infected mosquitoes and/or to continuously low background parasitemia. Overall, IgM antibody levels increased with the age of the donors and correlated well with a decrease in parasite densities (44). These antibodies in association with mononuclear cells may be involved in protection. Furthermore, in in vitro experiments, IgM antibodies appeared to be more effective than did IgG antibodies with respect to the reduction of parasite growth (3).

Specific IgE antibodies were recently detected in patients with malaria (10), but their level was low in relation to the sensitivity of the Western blotting technique, probably explaining our negative results. Furthermore, because of this poor sensitivity (compared with that of the enzyme-linked immunosorbent assay), we have not detected in individuals with malaria any autoantibodies which are known to react with normal RBC.

Age-related and season-related immune responses are the consequences of repeated infections. This amplification phenomenon following iterative stimulation by *P. falciparum* was reported for several antigens (5, 6, 11). The schizont and merozoite antigenic predominance is a well-described characteristic (21, 25, 27), but Western blotting data, expressed in histograms, appear to be more detailed (Fig. 8 and 9). As the latter data do not reflect the repartitioning of antibodies in each serum sample, individual variations cannot be accounted for. Since most of our studies were carried out with parasites cultured in vitro for many years, the results may not represent exactly what occurs in the field (19). The antigen set was slightly different from isolate to isolate, but the structural complexity of the natural isolate (Fig. 7A and

B), which has not been submitted to the selection pressure of adaptation in cultures, was not greater than that of the adapted *P. falciparum* isolate (Fig. 7C and D). These results indicate that most of the revealed antigens were not related to the geographic origin of the isolates, as previously reported (27, 40).

In summary, the technical improvements reported here allowed the analysis of about 200 serum samples at the same time and with similar conditions. Large numbers of antigenic molecules with various molecular masses were observed. This polymorphism corresponds to the wide repertoire of *P. falciparum* phenotypes because of the high rate of genetic rearrangement. However, a molecular mass determination is not sufficient to assess the identity of each polypeptide. Our data confirm the fact that specific antibody levels correlate positively with *P. falciparum* malaria exposure. Although natural immunity to malaria is not well understood, it is known to protect individuals from neurological attack. However, natural immunity does not prevent the appearance of parasites in the blood. Moreover, if children with similar parasite levels have had or have not had a malarial attack, factors other than a lack of immunity, such as ill health, malnutrition, and other infections, may be responsible.

ACKNOWLEDGMENTS

The helpful criticism of Ferrucio Santoro was much appreciated. We are grateful for the help of the staff of ORSTOM in Bobo-Dioulasso, Burkina Faso. We thank Annette Donadille for cultured parasites.

This study was supported by a grant from the ARC (French Cancer Research Association).

REFERENCES

1. **Bathia, A., P. Delplace, B. Fortier, J. F. Dubremetz, and A. Vernes.** 1987. Immunochemical analysis of a major antigen *Plasmodium falciparum* (P126) among ten geographic isolates. Am. J. Trop. Med. Hyg. **36**:15–19.
2. **Brown, G. V., J. G. Culvenor, P. E. Crewther, A. E. Bianco, R. L. Coppel, R. B. Saint, H. D. Stahl, D. J. Kemp, and R. F. Anders.** 1985. Localisation of the ring infected erythrocyte surface antigen (RESA) of *Plasmodium falciparum* in merozoites and ring infected erythrocytes. J. Exp. Med. **162**:774–779.
3. **Brown, J., B. M. Greenwood, and R. J. Terry.** 1986. Cellular mechanisms involved in recovery from acute malaria in Gambian children. Parasite Immunol. **8**:551–564.
4. **Bruce-Chwatt, L. J.** 1987. The challenge of malaria vaccine: trials and tribulations. Lancet **i**:371–373.
5. **Burkot, T. R., P. M. Graves, R. A. Wirtz, B. J. Brabin, D. Battistutta, J. A. Cattani, R. M. Maizels, and M. P. Alpers.** 1989. Differential antibody responses to *Plasmodium falciparum* and *P. vivax* circumsporozoite proteins in a human population. J. Clin. Microbiol. **27**:1346–1351.
6. **Campbell, G. H., F. H. Collins, A. D. Brandling-Bennett, I. K. Schwartz, and J. M. Roberts.** 1987. Age-specific prevalence of antibody to a synthetic peptide of the circumsporozoite protein of *Plasmodium falciparum* in children from three villages in Kenya. Am. J. Trop. Med. Hyg. **37**:220–224.
7. **Choumara, R., J. Hamon, J. Ricosse, H. Bailly, and D. Adam.** 1964. Le paludisme dans la zone pilote antipaludique de Bobo Dioulasso. Cah. ORSTOM Ser. Entomol. Méd. Parasitol. **1**:1–125.
8. **Coppel, R. L., P. E. Crewther, J. G. Culvenor, L. H. Perrin, G. V. Brown, D. J. Kemp, and R. F. Anders.** 1988. Variation in P126, a parasitophorus vacuole antigen of *Plasmodium falciparum*. Mol. Biol. Med. **5**:155–156.
9. **Delplace, P., J. F. Dubremetz, B. Fortier, and A. Vernes.** 1985. A 50 kilodalton exoantigen specific to the merozoite release-reinvasion stage of *Plasmodium falciparum*. Mol. Biochem. Parasitol. **17**:239–251.
10. **Desowitz, R. S.** 1989. *Plasmodium*-specific immunoglobulin E in sera from an area of holoendemic malaria. Trans. R. Soc. Trop. Med. Hyg. **83**:478–479.
11. **Esposito, F., S. Lombardi, D. Modiano, F. Zavala, J. Reeme, L. Lamizana, M. Coluzzi, and R. S. Nussenzweig.** 1988. Prevalence and levels of antibodies to the circumsporozoite protein of *Plasmodium falciparum* in an endemic area and their relationship to resistance against malaria infection. Trans. R. Soc. Trop. Med. Hyg. **82**:827–832.
12. **Freeman, R. R., and A. A. Holder.** 1983. Surface antigens of malaria merozoites. A high molecular weight precursor is processed to an 83,000 mol wt form expressed on the surface of *Plasmodium falciparum* merozoites. J. Exp. Med. **158**:1647–1653.
13. **Gazin, P., V. Robert, M. Cot, and P. Carnevale.** 1988. *Plasmodium falciparum* incidence and patency in a high seasonal transmission area of Burkina Faso. Trans. R. Soc. Trop. Med. Hyg. **82**:50–55.
14. **Good, M. F., L. H. Miller, and S. Kumar.** 1988. Limited immunological recognition of critical malaria vaccine candidate antigens. Science **242**:574–577.
15. **Ho, M., and H. K. Webster.** 1989. Immunology of human malaria. A cellular perspective. Parasite Immunol. **11**:105–116.
16. **Holder, A. A.** 1988. The precursor to major merozoite surface antigens: structure and role in immunity. Prog. Allergy **41**:72–97.
17. **Holder, A. A., R. R. Freeman, and S. C. Nicholls.** 1988. Immunization against *Plasmodium falciparum* with recombinant polypeptides produced in *Escherichia coli*. Parasite Immunol. **10**:607–617.
18. **Holder, A. A., M. J. Lockyer, K. G. Odink, J. S. Sandhu, V. Riveros-Moreno, S. C. Nicholls, Y. Hillman, L. S. Davey, M. L. V. Tizard, R. T. Schwarz, and R. R. Freeman.** 1985. Primary structure of the precursor to the three major surface antigens of *Plasmodium falciparum* merozoites. Nature (London) **317**:270–273.
19. **Howard, R. J., L. J. Panton, K. Marsh, I. T. Ling, E. J. Winchell, and R. J. M. Wilson.** 1986. Antigenic diversity and size diversity of *Plasmodium falciparum* antigens in isolates from Gambian patients. 1. S-antigens. Parasite Immunol. **8**:39–55.
20. **Jakobsen, P. H., T. G. Theander, J. B. Jensen, K. Molbak, and S. Jepsen.** 1987. Soluble *Plasmodium falciparum* antigens contain carbohydrate moieties important for immune reactivity. J. Clin. Microbiol. **25**:2075–2079.
21. **Kilejian, A.** 1980. Stage-specific proteins and glycoproteins of *Plasmodium falciparum*: identification of antigens unique to schizonts and merozoites. Proc. Natl. Acad. Sci. USA **77**:3695–3699.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
23. **Lambros, C., and J. P. Vanderberg.** 1979. Synchronization of *Plasmodium falciparum* erythrocyte stages in culture. J. Parasitol. **65**:418–420.
24. **Lyon, J. A., R. H. Geller, J. D. Haynes, J. D. Chulay, and J. L. Weber.** 1986. Epitope map and processing scheme for the 195,000-dalton surface glycoprotein of *Plasmodium falciparum* merozoites deduced from cloned overlapping segments of the gene. Proc. Natl. Acad. Sci. USA **83**:2989–2993.
25. **Marsh, K., L. Otool, R. J. Hayes, D. C. Carson, and B. M. Greenwood.** 1989. Antibodies to blood stage antigens of *Plasmodium falciparum* in rural Gambians and their relation to protection against infection. Trans. R. Soc. Trop. Med. Hyg. **83**:293–303.
26. **Mattei, D., K. Berzins, M. Wahlgren, R. Udomsangpetch, P. Perlmann, H. W. Griesser, A. Scherf, B. Müller-Hill, S. Bonnefoy, M. Guillotte, G. Langsley, L. Pereira Da Silva, and O. Mercereau-Puijalon.** 1989. Cross-reactive antigenic determinants present on different *Plasmodium falciparum* blood-stage antigens. Parasite Immunol. **11**:15–30.
27. **McBride, J. S., D. Walliker, and G. Morgan.** 1982. Antigenic diversity in the human malaria parasite *Plasmodium falciparum*. Science **217**:254–257.

28. **Merril, C. R., D. Goldman, S. A. Sedman, and M. H. Ebert.** 1981. Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science **211**:1437–1438.
29. **Miettinen-Bauman, A., W. Strych, J. McBride, and H. G. Heidrich.** 1988. A 46,000 dalton *Plasmodium falciparum* merozoite surface glycoprotein not related to the 185,000-195,000 dalton schizont precursor molecule: isolation and characterization. Parasitol. Res. **74**:317–323.
30. **Nkuo, T. K., and J. Deas.** 1987. Sera from Cameroon recognize proteins of *Plasmodium falciparum* isolates from geographically diverse areas of the world. Trans. R. Soc. Trop. Med. Hyg. **81**:891–895.
31. **Perrin, L. H., R. Dayal, and H. Rieder.** 1981. Characterization of antigens from erythrocytic stages of *Plasmodium falciparum* reacting with human immune sera. Trans. R. Soc. Trop. Med. Hyg. **75**:163–165.
32. **Peterson, M. G., R. L. Coppel, P. McIntyre, C. J. Langford, G. Woodrow, G. V. Brown, R. R. Anders, and D. J. Kemp.** 1988. Variation in the precursor of the major merozoite surface antigens of *Plasmodium falciparum*. Mol. Biochem. Parasitol. **27**:291–302.
33. **Quakyi, I. A., L. N. Otoo, D. Pombo, L. Y. Sugars, A. Menon, A. S. De Groot, A. Johnson, D. Alling, L. H. Miller, and M. F. Good.** 1989. Differential non-responsiveness in humans of candidate *Plasmodium falciparum* vaccine antigens. Am. J. Trop. Med. Hyg. **41**:125–134.
34. **Riley, E. M., S. Jepsen, G. Andersson, L. N. Otoo, and B. M. Greenwood.** 1988. Cell-mediated immune responses to *Plasmodium falciparum* antigens in adult Gambians. Clin. Exp. Immunol. **71**:377–382.
35. **Robert, V., P. Carnevale, V. Ouedraogo, V. Petrarca, and M. Coluzzi.** 1988. La transmission du paludisme humain dans un village de savane du Sud-Ouest du Burkina Faso. Ann. Soc. Belge Med. Trop. **68**:107–121.
36. **Robert, V., P. Gazin, C. Boudin, J. F. Molez, V. Ouedraogo, and P. Carnevale.** 1985. La transmission du paludisme en zone de savane arborée et en zone rizicole des environs de Bobo Dioulasso (Burkina Faso). Ann. Soc. Belge Med. Trop. **65**:201–214.
37. **Saul, A., P. Myler, L. Schofield, and C. Kidson.** 1984. A high molecular weight antigen in *Plasmodium falciparum* recognized by inhibitory monoclonal antibodies. Parasite Immunol. **6**:39–50.
38. **Thélu, J., and P. Ambroise-Thomas.** 1988. A septate polycarbonate cell culture unit used for *Plasmodium falciparum* and hybridomas. Trans. R. Soc. Trop. Med. Hyg. **82**:360–362.
39. **Thélu, J., and P. Ambroise-Thomas.** 1988. New prospects for the epidemiological and immunological study of malaria by Western blotting. C.R. Acad. Sci. **306**:11–16.
40. **Thélu, J., P. Ambroise-Thomas, B. Chumpitazi, and P. Kupka.** 1985. Purification and immunochemical study of *Plasmodium falciparum* exoantigens. J. Parasitol. **71**:542–546.
41. **Trager, W., and J. B. Jensen.** 1976. Human malaria parasites in continuous culture. Science **193**:673–675.
42. **Troye-Blomberg, M., and P. Perlmann.** 1988. T cell functions in *Plasmodium falciparum* and other malarias. Prog. Allergy **41**: 253–287.
43. **Wahlgren, M., K. Berzins, H. Perlmann, B. Wahlin, J. Carlsson, A. Björkman, P. Perlmann, L. A. McNicols, J. B. Dame, and T. F. McCutchan.** 1985. Pf 155, a vaccine candidate for protection against asexual stages of *Plasmodium falciparum* malaria, p. 51–56. *In* R. Lerner and R. Chanock (ed.), Modern approaches to vaccines. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. **Wahlgren, M., A. Björkman, H. Perlmann, K. Berzins, and P. Perlmann.** 1986. Anti-*Plasmodium falciparum* antibodies acquired by residents in a holoendemic area of Liberia during development of clinical immunity. Am. J. Trop. Med. Hyg. **35**:22–29.
45. **Weidanz, W. P., and C. A. Long.** 1988. The role of T cells in immunity to malaria. Prog. Allergy **41**:215–252.
46. **Winchell, E. J., I. T. Ling, and R. J. M. Wilson.** 1985. Stage specific production of S-antigens of *Plasmodium falciparum in vitro*. Trans. R. Soc. Trop. Med. Hyg. **79**:187–191.