CIRCUMSPOROZOITE PROTEINS OF HUMAN MALARIA PARASITES *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX**

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The malaria sporozoite injected into a host by the bite of the mosquito vector initiates the parasite cycle that culminates in clinical disease. This sporozoite stage is highly antigenic, and immunization with irradiated sporozoites has prevented the development of malaria in rodent and simian hosts as well as in several human volunteers (1). Antisporozoite antibodies detectable in the sera of the immunized primate hosts appear to be associated with immune resistance.

Recently, hybridoma-derived monoclonal antibodies directed against sporozoites of rodent and simian malaria were found to be protective, i.e., to abolish sporozoite infectivity both in vivo (2) and in vitro (3).[1] These antibodies react with circumsporozoite (CS)[2] proteins; that is, stage- and species-specific polypeptides that are uniformly distributed over the entire surface of the parasite and that are shed when cross-linked by antibodies (2, 4).[1]

The hybridoma technique was therefore used to develop monoclonal antibodies against sporozoites of *P. falciparum* and *P. vivax* in an attempt to identify the protective antigens of the human pathogens for their application in a vaccine.

Materials and Methods

Mice. BALB/c mice were immunized using viable and frozen sporozoites of *P. falciparum* or *P. vivax*. The *P. falciparum* sporozoites were of West African origin and were obtained by membrane feeding *Anopheles gambiae* mosquitoes on blood obtained from patients carrying *P. falciparum* gametocytes. *P. vivax* sporozoites for immunization were obtained from *Anopheles*

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[1] Cochrane, A. H., F. Santoro, V. Nussenzweig, R. W. Gwadz, and R. S. Nussenzweig. Monoclonal antibodies identify the protective antigens of sporozoites of *Plasmodium knowlesi*. Manuscript submitted for publication.

[2] *Abbreviations used in this paper:* CS, circumsporozoite; CSP, circumsporozoite precipitation reaction; IFA, indirect immunofluorescence; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

mosquitoes that had fed on *Aotus* monkeys infected with *P. vivax* of Southeast Asian origin (Indo I/CDC, Ong/CDC, and New Guinea/CDC, strains).

The *P. falciparum* and *P. vivax* sporozoite-immunized mice received seven and ten intravenous injections, respectively, totalling ~5.0 × 10^6 sporozoites. Spleens were removed from the immunized mice 3 d after the last booster, at a time when antisporozoite antibodies were detectable in their sera.

Hybridomas. Hybridomas were produced by fusing the spleen cells from the hyperimmunized mice with NS1 myeloma cells (5) using minor modifications of the technique of Kohler and Milstein (6).

The presence of antisporozoite antibodies in the hybridoma culture supernatant was detected by indirect immunofluorescence (IFA) using glutaraldehyde-fixed sporozoites of the appropriate species as antigen. Cells in IFA-positive wells were expanded, cloned by limiting dilution, and injected into pristane-primed CD2F1 mice to obtain larger quantities of ascites fluid and serum.

Monoclonal Antibodies. The antibody class of the hybridoma was determined by double diffusion in agar using the concentrated culture supernatants reacted against antisera specific for immunoglobulin class heavy and light chains (Litton Bionetics, Kensington, MD). Partial purification of the monoclonal antibody was carried out by precipitating pooled ascites fluid from the hybridoma-bearing mice in 50% ammonium sulfate. The precipitate was dissolved in phosphate-buffered saline and subjected to chromatography in a Sephadex G-200 column (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ). The antibody was eluted in the second optical density peak. Monovalent fragments were obtained by papain digestion (7).

The specificity of the monoclonal antibodies was determined using the circumsporozoite precipitation reaction (CSP) (8) and/or indirect immunofluorescence (9). The CSP reaction is a precipitate that forms at the posterior end of viable sporozoites after incubation with the appropriate antisporozoite antibody. The IFA assay was carried out using multiple well slides containing sporozoites fixed in 0.1% glutaraldehyde. After reaction with the monoclonal antibody, the washed slides were stained with fluorescein isothiocyanate-conjugated goat anti-mouse $F(ab')_2$ IgG (N. L. Cappel Laboratories, Inc., Cochranville, PA) using Evans Blue as a counterstain.

Parasite Species and Strains. The monoclonal antibodies were tested against sporozoites of human (*P. falciparum*, *P. vivax*), simian (*P. knowlesi*, *P. cynomolgi*), and rodent (*P. berghei*) malaria and also against *P. falciparum*-infected erythrocytes obtained from in vitro culture. *P. falciparum* sporozoites of West African (Gambia) and Southeast Asian (Thailand) origin were derived from human gametocyte carriers. Strains of *P. vivax* sporozoites for testing were obtained from mosquitoes fed on human or monkey hosts infected with Southeast Asian (Thailand, New Guinea/CDC, Ong/CDC, Indo I/CDC) or Central American (Sal I/CDC) vivax strains.

Sporozoite Neutralization Assay. The biological activity of the monoclonal antibody was determined by the sporozoite neutralization assay (10) in which viable sporozoites were incubated for 45 min at room temperature with Fab fragments or intact monoclonal antisporozoite antibodies. In most of the experiments, Fab fragments rather than the native molecules were used to assay for effects of the primary antigen-antibody reaction on parasite infectivity. As controls, an equal number of sporozoites were incubated in tissue culture Medium 199 (Gibco, Grand Island Biological Co., Grand Island, NY) or with Fab of a monoclonal antibody (3D11) directed against Pb44, the protective antigen of *P. berghei* sporozoites (2). After incubation, the sporozoites were injected intravenously into splenectomized chimpanzees. Blood smears were obtained at 2-d intervals and examined after Giemsa staining to determine the presence of a patent malaria infection. Chemotherapy was initiated as soon as patency was confirmed.

The chimpanzees were maintained in the Laboratory for Experimental Medicine and Surgery in Primates, New York University Medical Center, New York, or Yerkes Regional Primate Research Center, Emory University, Atlanta, GA.

Two neutralization experiments were performed with *P. falciparum*, one involving four, and the other, five chimpanzees. In both instances, the salivary glands of mosquitoes that fed on blood from patients from Thailand were the source of sporozoites. Before injection into the splenectomized chimpanzees, the sporozoites were incubated with 0.4–0.5 ml (2.5 mg) of the

specific or control monoclonals. In the first experiment, each chimpanzee received 2×10^4 sporozoites, and in the second, 1×10^4 sporozoites.

Two neutralization experiments with *P. vivax* were carried out in the same way with *P. vivax* from Thailand, using 2×10^4 sporozoites per animal. Experimental animals received sporozoites incubated with 0.4 ml (2.2 mg) of Fab fragments or intact monoclonal antibody directed against *P. vivax* sporozoites. Control chimpanzees received sporozoites incubated with either Fab of a nonrelated monoclonal antibody (2 mg) or tissue culture medium.

Immunoprecipitation of Sporozoite Antigens. Viable sporozoites of either *P. falciparum* (Thailand origin) or *P. vivax* (Ong/CDC strain) were incubated for 2–4 h in methionine-free RPMI 1640 to which was added 200 μCi of [^{35}S]methionine (New England Nuclear, Boston, MA) using the method of Yoshida et al. (11). Protease inhibitors were added to the labeled sporozoites before extraction with 1% Nonidet P-40 (N-P40; Particle Data Laboratories, Ltd., Cedarhurst, IL). The labeled sporozoite extracts were reacted overnight at 4°C with antibodies.

The antigen/antibody complexes were bound to *Staphylococcus aureus*, Cowan I strain (Pansorbin, Calbiochem-Behring, La Jolla, CA) (12), washed, and then eluted with sample buffer containing 2% sodium dodecyl sulfate (SDS), 20% β-mercaptoethanol, and 6 M urea. The eluates were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (13). The dried gels were stained with Coomassie Blue, treated with Enhance (New England Nuclear), and exposed to film (Kodak X-Omat AR, Eastman Kodak Co., Rochester, NY) for a variable period of time at −70°C before the fluorographs were developed.

As a source of antibodies to sporozoites, we used the monoclonal anti-*P. vivax* and anti-*P. falciparum* and, in addition, the sera of human volunteers who were protected against challenge with either sporozoites of *P. falciparum* (14) or *P. vivax* (15) after immunization by multiple exposures to the bites of irradiated malaria-infected mosquitoes. The volunteer protected against *P. falciparum* was immunized with and resistant to challenge by *P. falciparum* sporozoites of the Thau strain (Burma origin). He was also protected against challenge with sporozoites from other *P. falciparum* strains from Malaya, Panama, and the Philippines, but was not protected against *P. vivax* sporozoites (16). The volunteer who successfully resisted *P. vivax* sporozoite challenge had been immunized by exposure to the bites of irradiated mosquitoes infected with a Central American vivax strain (Gue., El Salvador) and the Chesson strain from New Guinea-South Pacific.

The sera of the immunized volunteers were kindly provided by Dr. Vincent McCarthy, University of Maryland Medical School. Additional immunoprecipitations were carried out using a monoclonal antibody directed against sporozoites of rodent malaria *P. berghei* (3D11) (3), two monoclonal antibodies directed against *P. knowlesi* sporozoites (2G3 and 8A8),[1] and normal serum from rodents and humans.

Results

Characterization of the Monoclonal Antibodies to P. vivax and P. falciparum. The single anti-*P. falciparum* sporozoite monoclonal antibody that we obtained was designated 3D6 and was identified as an IgG_1 immunoglobulin with a κ light chain. The anti-*P. vivax* sporozoite monoclonal antibody, designated 2F2, was identified as an IgG_{2b} immunoglobulin and also had a κ light chain.

Positive IFA reactions were obtained with concentrations as low as 0.3 μg/ml of purified 3D6 or 0.1 μg/ml of 2F2 when tested against glutaraldehyde-fixed sporozoites of the homologous species. As shown by the pattern of fluorescence, the monoclonals reacted with the entire surface membrane of the parasites. Neither monoclonal reacted in the IFA assay with sporozoites of simian (*P. knowlesi*, *P. cynomolgi*) or rodent (*P. berghei*) malaria, nor with the *P. falciparum* blood stage parasites.

Both monoclonals gave CSP reactions at high dilutions of serum (1:256) when incubated with viable sporozoites of the homologous but not heterologous species. The specific antigen detected on the surface of *P. falciparum* and *P. vivax* sporozoites by the respective monoclonal antibodies was shared by sporozoites of the same plasmodial

species, regardless of the geographic origin of the parasites. Thus, the anti-*P. falciparum* monoclonal that was derived from spleen cells of a mouse immunized with sporozoites of West African origin (Gambia) gave a high CSP titer when incubated with *P. falciparum* sporozoites obtained from Thailand. Similarly, the anti-*P. vivax* monoclonal raised using spleen cells from mice immunized with sporozoites of Southeast Asian strains gave CSP reactions with *P. vivax* sporozoites from Central America (El Salvador) as well as from Thailand (Table I).

To characterize the CS antigens involved in these reactions, extracts of sporozoites biosynthetically labeled with [^{35}S]methionine were immunoprecipitated with the monoclonals (Table I). In the case of [^{35}S]methionine-labeled *P. falciparum* (Thailand) sporozoites (Fig. 1), two specific bands of ~67,000 and 58,000 mol wt (Pf67 and Pf58) were precipitated by both the monoclonal antibody against *P. falciparum* sporozoites (3D6) (track b) and by the serum of the human volunteer immunized and protected against *P. falciparum* sporozoite challenge (track c). The Pf67 band appeared as a doublet and consists of two different polypeptides. In some experiments Pf58 was poorly labeled (Fig. 2). It appears that the amount of label incorporated into the low

TABLE I

Antibodies		Results of CSP and/or IFA reactions with sporozoites of				Specific immunoprecipitation from [^{35}S]methionine-labeled sporozoites of	
		P. falciparum		*P. vivax*		*P. falciparum*	*P. vivax*
Origin	Immunogen sporozoites of	West Africa	Southeast Asia	Central America	Southeast Asia	Southeast Asia	Southeast Asia
Hybridoma 3D6	*P. falciparum* (West Africa)	+	+	–	–	Pf 67 Pf 58	None
Hybridoma 2F2	*P. vivax* (S.E. Asia)	–	–	+	+	None	Pv 51 Pv 45
Hybridoma 2G3	*P. knowlesi* (H. strain)	+	+	–	–	Pf 67*	ND‡
Human serum	*P. falciparum* (S.E. Asia)	+	+	–	–	Pf 67 Pf 58 (Pf 80)§	(Pv 78)
Human serum	*P. vivax* (Central America and S. E. Asia)	–	–	+	+	(Pf 80)	Pv 51 Pv 45 (Pv 78)

* In this experiment, the immunoprecipitation of the *P. falciparum* extract by 3D6 and 2G3 yielded only one specific band, that is, Pf 67 (see Fig. 2 and text).

‡ Not done.

§ Antigens in parenthesis are not species specific: Pf80 was immunoprecipitated by human anti-*P. vivax* and Pv78 was immunoprecipitated by human anti-*P. falciparum* immune serum.

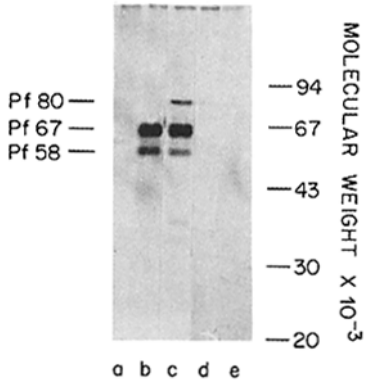

FIG. 1. Analysis of SDS-PAGE of [^{35}S]methionine-labeled *P. falciparum* (Thailand) sporozoite extract, immunoprecipitated with purified monoclonal anti-*P. falciparum* sporozoite antibody (3D6) (track b) or with serum obtained from a human volunteer immunized and protected against *P. falciparum* sporozoites (track c). The radiolabeled *P. falciparum* sporozoite extract was also reacted with serum of a human volunteer immunized against *P. vivax* sporozoites (track d) and a purified monoclonal antibody directed against *P. vivax* sporozoites (2F2) (track e). The results of immunoprecipitation with normal human serum are shown in track a.

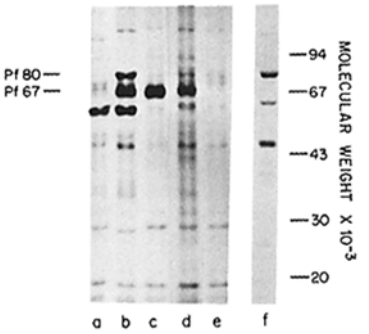

FIG. 2. [^{35}S]methionine-labeled *P. falciparum* (Thailand) sporozoites analyzed by SDS-PAGE after immunoprecipitation with purified monoclonal anti-*P. falciparum* sporozoite antibody (track c), antiserum obtained from a human volunteer immunized against *P. falciparum* sporozoites (track b), a cross-reacting monoclonal anti-*P. knowlesi* sporozoite antibody (2G3) (track d), or a non-cross-reacting monoclonal anti-*P. knowlesi* sporozoite antibody (8A8) (track e). A normal human serum control is shown in track a. Track f shows [^{35}S]methionine *P. falciparum* sporozoites immunoprecipitated with antiserum obtained from a human volunteer immunized against *P. vivax* sporozoites.

molecular weight protein depends on the viability of the parasites, but this could not be studied further because of the scarcity of *P. falciparum* sporozoites.

Pf67 is species specific because it is not recognized by monoclonals to *P. vivax* (Fig. 1, track e) or *P. berghei* (not shown in this figure) or by the serum of the human volunteer immunized with *P. vivax* (Fig. 1, track d; Fig. 2, track f). An unexpected finding was that one of the monoclonals raised against CS proteins of *P. knowlesi* sporozoites (2G3) that cross-reacts with *P. cynomolgi*,[1] also bound to the membrane of *P. falciparum*, as determined by CSP and IFA assays. As shown in Fig. 2 (track d), 2G3 also immunoprecipitated Pf67. Another monoclonal antibody to the CS proteins of *P. knowlesi* (8A8) did not cross-react with the *P. falciparum* sporozoites by IFA[1] and failed to immunoprecipitate Pf67 from the radiolabeled sporozoite extract (Fig. 2, track e). In addition to the Pf67 band, a polypeptide of 80,000 mol wt (Pf80) was immunopre-

cipitated by the sera of both the *P. falciparum*- and the *P. vivax*-immunized human volunteers (Fig. 2, compare track b and track f; Fig. 1, track c and track d, very faint).

The CS antigens of *P. vivax* sporozoites were also characterized by biosynthetically labeling the parasites with [^{35}S]methionine and immunoprecipitating the extract with monoclonal antibody 2F2 (Fig. 3, track d). The fluorograph of the SDS-PAGE revealed two major specific bands of apparent 45,000 (Pv45) and 51,000 (Pv51) mol wt. The Pv51 band is very broad and it may contain more than one polypeptide. A very similar pattern was obtained when the radiolabeled extract was immunoprecipitated with serum obtained from the human volunteer immunized and resistant to *P. vivax* sporozoite challenge (Fig. 3, track a).

Pv45 and Pv51 are species specific and were not precipitated by a monoclonal antibody directed against *P. berghei* (3D11) (Fig. 3, track e) or by the monoclonal antibody directed against *P. falciparum* sporozoites (3D6) (not shown in Fig. 3), or by the serum of the volunteer immunized and protected against *P. falciparum* sporozoites (Fig. 3, track b). As was the case with *P. falciparum* sporozoites, an additional polypeptide (Pv78) that is not species specific can be precipitated from the *P. vivax* extract by both the serum of the *P. vivax*- and the *P. falciparum*-immunized human volunteer.

Neutralization of Sporozoites of P. falciparum and P. vivax by Monoclonal Antibodies. The monoclonal 3D6 was tested for its ability to neutralize the infectivity of *P. falciparum* sporozoites as described in the Materials and Methods section. Two separate experiments were carried out in chimpanzees (Table II).

In experiment I, the two control chimpanzees that received 2×10^4 *P. falciparum*

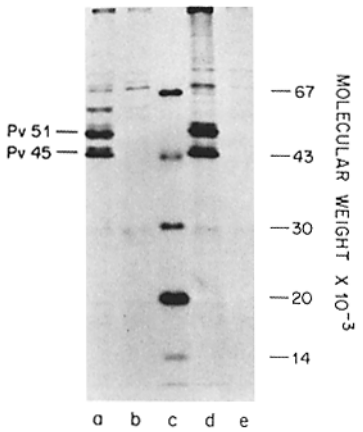

FIG. 3. Analysis by SDS-PAGE of proteins immunoprecipitated from a [^{35}S]methionine-labeled extract of *P. vivax* sporozoites (Ong/CDC strain) by antiserum obtained from a human volunteer immunized against *P. vivax* sporozoites (track a), a human volunteer immunized against *P. falciparum* sporozoites (track b), serum of CD2F1 mice bearing a hybridoma against *P. vivax* sporozoites (2F2) (track d), or serum of CD2F1 mice bearing a hybridoma against sporozoites of rodent malaria *P. berghei* (3D11) (track e). Track c contains radiolabeled molecular weight markers. The heavy background observed in track d appears to be the result of nonspecific trapping of radiolabeled material in the monoclonal precipitate. The monoclonal anti-*P. vivax* antibody (2F2) precipitated ~7.7% of the radiolabeled vivax extract as compared to only 1.3% of the counts being precipitated by 3D11. Subsequent gels have shown that this nonspecific background can be eliminated by preabsorption of the labeled sporozoite extract with Staph A before immunoprecipitation with the monoclonal antibody.

TABLE II

Sporozoite Neutralization Experiments Using Monoclonal Antisporozoite Antibodies

Experiment	Chimpanzees injected with sporozoites of	Experimentals, first day of patency	Controls, first day of patency
I*	*P. falciparum*	21, not patent‡	12, 14
II§	*P. falciparum*	21, 25	14, 14, 14
III‖	*P. vivax*	36, not patent¶	17, 21
IV**	*P. vivax*	21, 28, 33	19, 19, 24

* Each animal received 2×10^4 *P. falciparum* (Thailand) sporozoites. The two experimental chimpanzees received parasites incubated with Fab of 3D6 (2.5 mg). The two controls were injected with sporozoites incubated with Fab of 3D11 (2.5 mg) or tissue culture medium, respectively.

‡ Experiment terminated on day 61.

§ Same experimental conditions as in experiment I, except that each animal received 1×10^4 sporozoites. Controls received parasites incubated in tissue culture medium.

‖ All chimpanzees were injected with 2×10^4 *P. vivax* (Thailand) sporozoites. Experimental animals received sporozoites incubated in Fab of 2F2 (2.2 mg). Control was tissue culture medium.

¶ Experiment terminated on day 79.

** Conditions are the same as in experiment III, except that the third experimental chimpanzee received sporozoites incubated with intact 2F2. The first and the third control chimpanzees received sporozoites incubated with Fab of 3D11 (2 mg), while the second received parasites in tissue culture medium.

sporozoites incubated either in Fab fragments of a monoclonal antibody directed against *P. berghei* sporozoites (3D11) or in Medium 199 became patent 12 and 14 d post-inoculation, respectively. Of the two chimpanzees injected with the *P. falciparum* sporozoites incubated with Fab fragments of the monoclonal anti-*P. falciparum* antibody (3D6), one developed a patent infection 21 d post-injection. The second recipient of 3D6 remained negative until the experiment was terminated 61 d after the injection of parasites.

In the second experiment, the three control chimpanzees that received 1×10^4 sporozoites incubated in tissue culture medium, showed patent infections 14 d post-injection. The two experimental animals injected with the sporozoites incubated in Fab fragments of monoclonal 3D6 showed delayed prepatent periods of 21 and 25 d.

Two sporozoite-neutralizing experiments, involving a total of 10 splenectomized chimpanzees, were carried out to test the ability of the monoclonal anti-*P. vivax* antibody (2F2) to decrease sporozoite infectivity. In the first experiment, two control chimpanzees, each injected with 2×10^4 viable *P. vivax* (Thailand) sporozoites incubated in Medium 199, developed vivax malaria 17 and 21 d post-inoculation. Of the two chimpanzees that received sporozoites incubated with the Fab fragments of the monoclonal anti-*P. vivax* antibody (2F2), one had a delayed prepatent period of 36 d, while the other remained negative.

The second vivax experiment was carried out using six splenectomized chimpanzees, each injected with 2×10^4 *P. vivax* (Thailand) sporozoites. Two controls that received sporozoites incubated with Fab of 3D11 became infected 19 and 24 d post-inoculation. The third control, injected with *P. vivax* sporozoites incubated with tissue culture medium, was patent 19 d after injection. Two experimental chimpanzees inoculated with sporozoites incubated with Fab of 2F2 had detectable parasitemia 21 and 28 d post-inoculation. The third experimental animal, which received sporozoites incubated with intact molecules of 2F2, developed vivax malaria 33 d after injection.

Discussion

One of the main findings of the present paper is that the interaction of monoclonal antibodies 3D6 and 2F2 with CS proteins of *P. falciparum* and *P. vivax* diminishes the infectivity of the parasites.

In two separate experiments (Table II) involving a total of nine chimpanzees injected with 1–2 × 10^4 *P. falciparum* sporozoites, the five control animals became patent between 12 and 14 d after the injection, whereas in the experimental group, one animal failed to develop patency, and in the three others the prepatent periods were 21, 21, and 25 d. Very similar results were obtained in 10 chimpanzees injected with 2 × 10^4 *P. vivax* sporozoites. The four controls became patent between 17 and 24 d, whereas in the experimental group, one animal did not become infected and the other four had prepatent periods of 21, 28, 33, and 36 d.

Although complete neutralization was not achieved, these results are encouraging for several reasons. In the first place, 3D6 and 2F2 are the first and only monoclonals that we have raised against the CS proteins of *P. falciparum* and *P. vivax*. The delay in patency observed in the experimental animals suggests that a large proportion of the injected sporozoites might have been neutralized by the antibodies because in experimental models of malaria the dose-response curve relating the number of injected sporozoites to the prepatent period is very flat. For example, in several experiments, a variation of <1 d in the prepatent period occurred in groups of mice injected with 10^3 or 5 × 10^3 *P. berghei* sporozoites (17). Also, no significant variation in prepatent period was observed in monkeys injected with between 10^3 or 5 × 10^4 sporozoites of *P. cynomolgi* (18).

Because monovalent fragments of monoclonal antibodies to CS proteins have neutralizing activity, it is very likely that the effect observed is a direct consequence of the primary antigen-antibody reaction rather than of secondary effects in vivo, which could lead to the elimination of parasites such as complement-mediated lysis or phagocytosis. In rodent and monkey malaria models, Fab fragments of CSP-inducing monoclonals also neutralize their infectivity, suggesting that the corresponding CS proteins play a role in parasite infectivity. In fact, Fab fragments to Pb44, the *P. berghei* CS antigen, inhibit adhesion and intracellular development of the parasite in target cells in vitro (19).

The species specificity but not strain specificity of the antibodies to the CS proteins of *P. vivax* and *P. falciparum* (Table I) is consistent with the earlier data on the characteristics of the humoral response and protective immunity to sporozoites (1). An additional important observation was that the *P. falciparum* of Southeast Asian origin used to inject the chimpanzees was neutralized by monoclonal antibodies raised against a West African isolate of the parasite.

Both the monoclonals 3D6 (anti-*P. falciparum*) and 2F2 (anti-*P. vivax*) immunoprecipitated two antigens of different molecular weight (Pf67 and Pf58; and Pv51 and Pv45) from extracts of the homologous parasites metabolically labeled with [^{35}S]methionine. Pf67 and Pf58 are structurally related because their tryptic peptide maps obtained by high performance liquid chromatography on C18 columns are very similar, if not identical (F. Santoro, A. Cochrane, E. H. Nardin, R. Gwadz, R. Nussenzweig, V. Nussenzweig, and A. Ferreira, manuscript in preparation). The relationship between each pair of polypeptides and the corresponding membrane-associated CS proteins has not been studied because of the difficulties in obtaining *P.*

vivax and *P. falciparum* sporozoites. It should be pointed out, however, that cytoplasmic precursors of CS proteins have been found both in *P. berghei* and *P. knowlesi* (11).[1]

The same extracts of metabolically labeled sporozoites were immunoprecipitated by the serum of human volunteers vaccinated with x-irradiated sporozoites of *P. vivax* or *P. falciparum* and protected against infection with the homologous parasite. It is remarkable that the CS proteins of *P. vivax* and *P. falciparum* were the main polypeptides specifically recognized by these sera (Figs. 1 and 3). The CS proteins are also among the few polypeptides that can be immunoprecipitated from crude extracts of *P. berghei* and *P. knowlesi* sporozoites by the serum of vaccinated mice or monkeys (3).[1] The present and previous findings indicate, therefore, that CS proteins are among the most potent immunogens of sporozoites.

In addition to the CS proteins of *P. vivax* and *P. falciparum*, other polypeptides (Pf80, Pv78) are recognized by the serum of both human volunteers. These antigens are probably not membrane associated because the sera of the human volunteers do not cross-react with membrane antigens of heterologous species as shown by IFA and CSP reactions (Table I).

Of interest is the observation that the monoclonal anti-CS protein of *P. knowlesi* (2G3) cross-reacted with the CS protein of *P. falciparum*. The fact that this monoclonal neutralized the infectivity of *P. knowlesi* for monkeys[1] lends additional support to the data in the present paper indicating that the CS proteins of *P. falciparum* and *P. vivax* can be the target of protective antibodies. Moreover, recent studies using monoclonals have also revealed the presence of cross-reactive epitopes in CS proteins of rodent (*P. berghei*, *P. yoelii nigeriensis*) (N. Yoshida, R. Nussenzweig, and V. Nussenzweig, manuscript in preparation) and simian (*P. knowlesi*, *P. cynomolgi*)[1] malarias. Taken together, these observations suggest that CS proteins might belong to a family of related surface molecules with shared as well as species-specific structural features and a common function perhaps essential for the development and penetration of the parasite into the target cell. The apparent simplicity and conservation of the antigenic repertoire of the CS proteins should facilitate the immunoprophylaxis of malaria based on a sporozoite vaccine.

Summary

Monoclonal antibodies were raised against sporozoites of two species of malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax*. The antibodies reacted with polypeptides (circumsporozoite proteins) that are uniformly distributed over the entire surface of sporozoites, as shown by indirect immunofluorescence and by the circumsporozoite precipitin reaction. The epitopes recognized by the monoclonal antibodies were expressed on sporozoites from different geographical isolates of the homologous species but were not detected on sporozoites of heterologous species nor on blood forms of the parasite.

The monoclonal antibody to *P. falciparum* specifically immunoprecipitated two polypeptides of apparent 67,000 mol wt (Pf67) and 58,000 mol wt (Pf58) from extracts of [^{35}S]methionine-labeled *P. falciparum* sporozoites. Similarly, the anti-*P. vivax* monoclonal immunoprecipitated two proteins of 51,000 mol wt (Pv51) and 45,000 mol wt (Pv45) from extracts of metabolically labeled *P. vivax* sporozoites. The extracts were also reacted with the serum of human volunteers successfully vaccinated with sporo-

zoites of either *P. vivax* or *P. falciparum*. The patterns of immunoprecipitation were almost identical to those obtained with the corresponding monoclonal antibodies.

The circumsporozoite proteins of *P. falciparum* and *P. vivax* play a role in immune protection. Incubation of the appropriate monoclonal antibody with viable sporozoites of the homologous species significantly reduced parasite infectivity, as determined by sporozoite neutralization assays carried out in splenectomized chimpanzees.

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