Bulletin of the World Health Organization, 57 (Suppl. 1). 211-217 (1979)

Characterization of sporozoite surface antigens by indirect immunofluorescence: detection of stage- and species-specific antimalarial antibodies

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*Indirect immunofluorescence (IF) was used to localize stage-specific antigen(s) on the surface of the sporozoite membrane. The authors examined the feasibility of using an IF assay to determine whether an antisporozoite response is developed by individuals living in endemic areas. The specificity and sensitivity of the IF assay were first defined by using hyperimmune sera of sporozoite-immunized hosts protected against rodent (*P. berghei*), simian (*P. knowlesi*), and human (*P. falciparum, P. vivax*) malarias. Species- and stage-specific antisporozoite antibodies were detected by this technique when viable or glutaraldehyde-fixed sporozoites were used as antigen. The sensitivity of indirect immunofluorescence was found to be 5-10 times greater than that of the circumsporozoite precipitation (CSP) assay. Preliminary studies on sera obtained from individuals living in endemic areas showed that the IF assay can be used to detect species- and stage-specific antisporozoite antibodies in sera of naturally-infected human and simian hosts.*

Immunization of mammalian hosts with either mosquito or blood stages of malarial parasites results in a large measure of protection against infection, which has been shown to be strictly stage- and species-specific (*1, 2, 3*). Sporozoite-immunized rodents, primates, and human volunteers, while totally resistant to challenge with the homologous stage of the parasite, remain fully susceptible to blood-induced infection (*2, 4*). The reverse is also true; i.e., primates and rodents immunized and resistant to blood stages, still develop numerous liver stages upon challenge with sporozoites of the same malarial species (*5*).

This stage and species specificity is also reflected in the antibody response of sporozoite-immunized rodents (*6*), nonhuman primates (*7*), and humans (*4, 8*), as detected by the circumsporozoite precipitation (CSP) reaction. Conversely, the sera of animals infected or immunized with erythrocytic stages of plasmodia do not produce a positive CSP reaction.

While the results of active immunization and certain serological findings demonstrate the presence of antigens specific to each stage of the parasite's life cycle, other evidence, obtained primarily by immunofluorescence (IF), indicates that different developmental phases of the same parasite also possess shared antigens. In fact, avian antisporozoite antisera were shown by direct immunofluorescence to interact with infected red blood cells (IRBC) (*9*) as well as with sporozoites of *Plasmodium gallinaceum* (*10*). Conversely, antisera raised against RBCs infected with avian and rodent plasmodia have been employed to stain sporozoites by both direct and indirect immunofluorescence (*11, 12*).

Since stage-specific and shared antigens have been shown to exist, it was important to establish sensitive methods that would permit the discrimination of the stage-specific from the shared antigens. This is relevant not only for seroepidemiological purposes, but also to provide the tools to localize and characterize the corresponding sporozoite-specific antigens, including those responsible for protection. The present report summarizes our data on sporozoite-specific surface antigens, obtained primarily through the use of fluorescein-conjugated antisera interacting with intact sporozoites.

SPOROZOITE-SPECIFIC SURFACE ANTIGENS

Since ultrastructural findings showed a surface deposition of immunoglobulin on sporozoites incubated in immune serum (*13*), it was postulated that intact sporozoites would, in suspension, react exclusively with antisporozoite antisera. It was thought that earlier observations of cross-reaction of sporozoites

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with anti-IRBC antisera might have been due to the air-drying and freezing of the parasites. This procedure could conceivably have exposed internal shared antigens responsible for the cross-reactions.

We therefore compared the results of IF obtained by using viable and fixed sporozoites, as well as air-dried, freeze-thawed preparations of sporozoites and IRBC, exposing them to a series of antisera of well-defined specificity (*14*). The stage specificity of the antimalarial antibodies detected with sporozoites and IRBC of *P. knowlesi* is summarized in Table 1. The antisporozoite antiserum used in these assays was obtained from a monkey that resisted challenge with viable sporozoites upon immunization with multiple doses of irradiated sporozoites of *P. knowlesi*. The "anti-IRBC and gametes" antiserum had been obtained from a single rhesus monkey that received multiple doses of parasitized blood, containing gametes, trophozoites and schizonts of *P. knowlesi*, administered in Freund's complete adjuvant (FCA). The anti-merozoite antiserum was obtained from a protected monkey that had received 3×10^9 semipurified merozoites emulsified in FCA. After interaction with the antiplasmodial antisera, all preparations were stained with fluorescein isothiocyanate (FITC) conjugated sheep antihuman IgG (Behring Laboratories) using Evans blue as counterstain.

Table 1. Stage specificity of antimalarial antibodies as detected with sporozoites and blood forms of *P. knowlesi*

Parasite stage used for production of antisera in rhesus monkeys	Sporozoites				IRBC[a]
	Viable		Fixed[b]	Air-dried	Air-dried
	CSP	IF	IF	IF	IF
Sporozoites	1 4	1 256	1:1024	1·256	1·64
IRBC[a] and gametes	neg	neg.	neg	1 64	1:4096
Merozoites	neg	neg	neg	1.64	1 4096
Control: normal rhesus serum	neg	neg	neg	neg	neg

[a] Infected red blood cells.

[b] Sporozoites fixed with 1 g/litre (0 1%) glutaraldehyde.

As can be seen in Table 1, viable as well as glutaraldehyde-fixed sporozoites reacted exclusively with the antisporozoite antiserum. Two anti-IRBC sera, with high IF titres (1:4096) against *P. knowlesi* IRBC, failed to give positive CSP or IF reactions with viable or glutaraldehyde-fixed sporozoites. However, once the parasites were disrupted by air-drying and freezing, significant serological cross-reactions between the sporozoites and the anti-IRBC antisera were detected.

Similar results were observed in the rodent–*P. berghei* system. Sera from mice with acute or chronic blood-induced *P. berghei* infections with IF titres of 1:1280 against IRBC, failed to cross-react with viable sporozoites in both the CSP and the IF assays. It was only when the sporozoites were air-dried and frozen that serological cross-reactions between blood and sporozoite stages were observed.

Therefore, we believe that the target of the anti-sporozoite antibody is a surface antigen. This concept is supported by the finding that the stage specificity of the IF reaction is maintained with glutaraldehyde-fixed sporozoites. Sporozoites of *P. knowlesi* were fixed in 1 g/litre (0.1%) glutaraldehyde in 0.1 mol/litre cacodylate buffer and 40 g/litre (4%) sucrose solution for 10 min at room temperature. The stage and species specificity of the reactions with glutaraldehyde-fixed sporozoites were identical with those of the reactions performed with viable sporozoites. The fixed *P. knowlesi* sporozoites can be stored for approximately one month at 4 °C and over 6 months at −70 °C without loss of reactivity. Glutaraldehyde fixation of *P. berghei* sporozoites, however, results in some background fluorescence when they are allowed to interact with saline or normal mouse serum. Correction for these low false positive reactions (1:4) must be made when fixed *P. berghei* sporozoites are used in serological assays.

Studies on the biochemical nature of the sporozoite surface antigen(s) are at present in progress in our laboratory. Surface membrane components of intact sporozoites of rodent (*P. berghei*) and simian (*P. knowlesi*) malaria have been labelled by lactoperoxidase catalysed ^{125}I-iodination (*15*). These parasites were then disrupted and the corresponding extracts analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by autoradiography. In the case of sporozoites of *P. berghei*, this yielded 6 main labelled bands (see the paper by Gwadz et al., pp. 165-173). Sera from sporozoite-immunized mice precipitated only one of the main labelled components of the parasite extract. This was clearly observed upon gel electrophoresis followed by autoradiography of the immunoprecipitates. Since this method iodinates tyrosine residues of surface components of cell membranes, we believe we have identified a proteinaceous surface antigen.

Whether this surface antigen is a glycoprotein, similar to those present on the surface coat of several other protozoan parasites, such as *Leishmania* spp (*16, 17*), *Entamoeba histolytica* (*18*), and *Trypanosoma cruzi* (*19*), remains to be determined. These protozoa were shown to agglutinate upon interaction with concanavalin A and other lectins. Our attempts to demonstrate a carbohydrate moiety on the surface of viable *P. berghei* and *P. knowlesi* sporozoites,

using fluorescein-conjugated concanavalin A, wheat germ agglutinin and soybean agglutinin (Miles-Yerba Research Laboratories, Elkhart, Indiana, USA) have thus far been unsuccessful.

OCCURRENCE OF SPOROZOITE-SPECIFIC ANTIGENS DURING THE PARASITE'S LIFE CYCLE

The development of stage-specific surface antigens during the course of sporozoite maturation was studied by comparing oocyst sporozoites (11 days) with salivary gland sporozoites (20 days) as antigens in the IF and CSP reactions. Earlier experiments had shown that oocyst sporozoites failed to give CSP reactions when incubated with antisera raised against salivary gland parasites, in rodent (*20*) and simian malaria (*7*). However, ultramicroscopic observations have demonstrated a small amount of surface deposition on oocyst sporozoites incubated with antisera produced by immunization with salivary gland sporozoites (*13*).

In the present study, *P. knowlesi* sporozoites obtained from 11-day-old oocysts failed to produce a clearly positive CSP reaction, even with undiluted antiserum. Twenty-day-old sporozoites obtained from the salivary glands of the same group of mosquitos gave a CSP titre of 1:64 with the same antiserum. However, using the more sensitive indirect immunofluorescent technique, we detected weak positive reactions on 11-day-old oocyst parasites at somewhat lower titres than those obtained with mature 20-day-old sporozoites. The young sporozoites thus appear to have an incomplete antigenic repertoire rather than a total lack of sporozoite surface antigen, as had been suggested on the basis of earlier CSP findings.

It is of interest that air-dried preparations of 11-day-old oocyst parasites failed to react with either antisporozoite or anti-IRBC-stage antisera. This contrasts sharply with the results obtained with air-dried preparations of salivary gland sporozoites, which gave an IF titre of 1:16 384 with the same antisporozoite antiserum. Thus, antigenic determinants on the immature sporozoite appear to be less stable.

We recently investigated the persistence of sporozoite-specific antigens during maturation and growth of exoerythrocytic forms (EEF) of *P. berghei* within hepatocytes (*21*). During the early phase of development after the inoculation of sporozoites into a vertebrate host, sporozoite-specific antigens are present in the EEF. In fact, young EEF, obtained up to 16 h after sporozoite inoculation, react well with antisporozoite but only weakly with anti-IRBC antisera. From 18 to 30 hours after sporozoite inoculation there follows a period of EEF development in which these parasites produce a positive IF reaction with both antisporozoite and anti-IRBC antisera. Later during the life cycle, i.e., 36–42 or more hours after sporozoite inoculation, the EEF react exclusively with anti-IRBC antisera, and are not recognized by stage-specific antisporozoite antibodies.

SPECIES-SPECIFIC SPOROZOITE ANTIGENS

Both protection and the humoral responses of sporozoite-immunized rhesus monkeys and humans have been found to be species- as well as stage-specific. This species specificity of the antibody response was established several years ago by performing the CSP assay with sera obtained from animals immunized with sporozoites of simian and human malaria (*7, 22*). The sera of human volunteers immunized with irradiated sporozoites of *P. falciparum* and *P. vivax* presented this same species-specificity in the CSP reaction (*23, 24*). This contrasts with the findings in rodent malarias, in which immunization with sporozoites of *P. berghei* and *P. vinckei vinckei* and *P. chabaudi chabaudi* resulted in cross-protection against challenge with sporozoites of all the rodent malarial species. The sera of these immunized animals cross-reacted in the CSP assay with sporozoites of all the other rodent malarias. However, they failed to give a positive CSP reaction with sporozoites of simian and avian malaria.

Recently the question of species- and strain-specificity of sporozoite antigens was re-examined by using the IF assay. Viable and glutaraldehyde-fixed sporozoite preparations react only with species-specific antisporozoite antisera (Table 2). This strict species specificity of the antisporozoite antibody response, as detected by IF, is in marked contrast with the well known extensive degree of cross-reactivity found when IRBC are used as antigen in the IF assay. Freezing of the sporozoites prior to their interaction with the antisera did not cause the loss of species-specificity.

With regard to the strain-specificity of antisporozoite antibodies, earlier studies based on the CSP reaction had indicated that there were extensive cross-reactions between different geographical isolates or strains of *Plasmodia* (*22, 23*). Our initial studies using the sera of sporozoite-immunized human volunteers indicated that the IF technique also detects cross-reactions between various geographical isolates of the same malaria species.

The findings that immunization of human volunteers with irradiated sporozoites of *P. falciparum* and *P. vivax* led to protection against challenge with different strains of the homologous species of sporozoites, emphasizes the importance of serological cross-reactivity (*8, 23, 24*).

Table 2. Species specificity of antisporozoite antibodies as detected by immunofluorescence using glutaraldehyde-fixed sporozoites

Strain of sporozoite used for production of antisera	Glutaraldehyde-fixed sporozoites			
	P. knowlesi (Phil strain)	*P. cynomolgi* (B strain)	*P. vivax* (India strain)	*P. berghei* (NK 65 strain)
P. knowlesi (Phil strain)	pos.	neg	neg	neg
P. cynomolgi (B strain)	neg	pos	neg	neg
P. vivax (Chesson strain)	neg	neg	pos.	neg.
P. falciparum (Burma strain)	neg	neg	neg	neg.
P. berghei (NK 65 strain)	neg	neg	neg	pos.

However, we have recently detected what appears to be an exception to the earlier findings of extensive serological cross-reactivity between sporozoites of different geographical isolates. As can be seen in Table 3, viable sporozoites of the H strain of *P. knowlesi* completely failed to interact, in both the CSP and the IF assays, with antisera obtained from rhesus monkeys and mice immunized with sporozoites of a Philippine isolate (Phil strain) of *P. knowlesi*. The reverse was also true, i.e., sporozoites of the Phil strain completely failed to react with anti-H strain antisera. These antisera gave high titres with the homologous sporozoites, in both the CSP and IF reactions.

Table 3. Failure to detect cross-reactions between H and Philippine strains of *P. knowlesi* when using viable sporozoites

Strain used for production of antisera [a]	*P. knowlesi* H. strain viable sporozoites		*P. knowlesi* Phil strain viable sporozoites	
	CSP	IF	CSP	IF
H strain sporozoites [b]	1 64	1 1024	neg	neg
H strain sporozoites [c]	1 64	1 1024	neg.	neg.
H strain IRBC [b]	neg.	neg.	neg	neg.
Phil strain sporozoites [b]	neg.	neg.	1 64	1:1024
Phil strain sporozoites [c]	neg	neg	1·64	1 256
Controls.				
P. cynomolgi sporozoites [b]	neg	neg	neg	neg.
Normal rhesus serum	neg	neg	neg	neg

[a] Same antisera used in the experiments summarized in Tables 3 and 4
[b] Rhesus monkey.
[c] Mouse.

Our initial hypothesis was that the Philippine and H strains, although sharing the same morphology and producing the same periodicity and course of infection in *Macaca mulatta*, might perhaps be separate plasmodial species. Further work, however, revealed cross-reactions that were not compatible with the hypothesis of separate species. As can be seen in Table 4, cross-reactions between H and Philippine strain sporozoites became detectable with both glutaraldehyde-treated and air-dried sporozoite preparations, indicating that these strains share a common *P. knowlesi* species antigen. Weak, but clearly positive IF reactions were seen when glutaraldehyde-fixed sporozoites of the H strain were treated with two anti-Phil strain sera of different origins. The reverse did not occur, i.e., glutaraldehyde-fixed sporozoites of the Phil strain failed to give a positive IF reaction with two anti-H strain sera obtained from different animals. The use of air-dried sporozoites detected cross-reactions with both parasite strains.

Table 4. Detection of cross-reactions between the H and Philippine strains of *P. knowlesi* when using air-dried and fixed sporozoites

Strain of sporozoite used for production of antisera	*P. knowlesi* H. strain sporozoites		*P. knowlesi* Phil strain sporozoites	
	Fixed IF	Air-dried IF	Fixed IF	Air-dried IF
H strain sporozoites [a]	1 16 384	1 4096	neg	1:64
H strain sporozoites [b]	1 16 384	1:4096	neg	1 64
H strain IRBC [a]	neg	1 64	neg	1.64
Phil strain sporozoites [a]	1 256W [c]	1 16	1:16 384	1.4096
Phil strain sporozoites [b]	1:64W [c]	1.64	1 16 384	1·4096
Controls				
P. cynomolgi sporozoites [a]	neg	neg	neg.	neg
Normal rhesus serum	neg	neg	neg.	neg

[a] Rhesus monkey
[b] Mouse
[c] Weak reactions at all serum dilutions

One interpretation of these results is that there might be quantitative differences in the amount of antigen(s) expressed on sporozoites of the Philippine as compared with the H strain of *P. knowlesi*. The results would also suggest the presence of more than one surface antigen—one that is recognized only by strain-specific antibodies, and produces high titres of CSP and IF reaction, and possibly a second one that is shared by both strains. The extent to which these differences in sporozoite antigen(s) are relevant to protection will be clarified by our current experiments with rhesus monkeys, using sporozoites of both of these *P. knowlesi* strains.

ANTISPOROZOITE ANTIBODY RESPONSE IN NATURALLY INFECTED AND IMMUNIZED HOSTS

An earlier attempt, by using the CSP reaction, to detect antisporozoite antibodies in the sera of individuals living in malaria-endemic areas of Africa has given negative results (*25*). Since CSP is not a very sensitive reaction and is not capable of detecting low titres of antisporozoite antibodies, we decided to reinvestigate this question by using the more sensitive IF test (*14*).

The search for antisporozoite antibodies in the sera of people living in malaria-endemic areas was initiated by us last year at the Medical Research Council Laboratories in The Gambia, West Africa, with the cooperation of Dr R. S. Bray, then Director of that Institute, together with Dr R. Carter of the Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD. Sporozoites were obtained by infecting *Anopheles gambiae* mosquitos through membrane feeding on *P. falciparum*-infected blood, obtained from patients at the Medical Research Council clinic. Owing to numerous technical problems, only 4 Gambian sera could be tested. These serum samples were from patients ranging in age from 6 months to 73 years, all with positive IF titres against *P. falciparum*-infected RBC. All 4 sera gave a positive IF reaction with viable sporozoites of *P. falciparum* (Table 5). Owing to the small number of sporozoites available, we were not able to perform accurate titrations of this antisporozoite antibody response. Controls for specificity included the serum from a volunteer immunized with sporozoites of *P. vivax* (*24*), an anti-*P. knowlesi* sporozoite antiserum, and normal human sera obtained from the New York City Blood Bank. All these sera gave negative reactions with the viable *P. falciparum* sporozoites.

In contrast to the low sporozoite antibody titres in the four Gambian patients, the serum of a volunteer who was immunized and had become protected against *P. falciparum* sporozoite challenge (*4*), had an IF titre of 1:4096 and a positive CSP reaction.[a] When this serum sample was tested against *P. falciparum*-infected RBC, only a weak, nonsignificant reaction was noted. However, another serum sample collected after this volunteer had succumbed to a challenge with blood stages of *P. falciparum* gave an anti-IRBC titre of 1:1024. Repeated absorption of this serum with *P. falciparum*-infected RBC removed the antibodies directed at the blood stages of this parasite. but did not alter the antisporozoite IF reaction.

We also examined the sera of feral monkeys (*Macaca fascicularis*) recently imported from Malaysia and Indonesia, areas endemic for *P. knowlesi* and *P. cynomolgi* malaria (*26*). Of these sera, 36% gave positive IF reactions against erythrocytes infected with *P. knowlesi*. All the sera were CSP negative with viable sporozoites of *P. knowlesi*. All the sera (10/10) of the Indonesian group of animals gave negative IF reactions when tested against viable *P. knowlesi* sporozoites. However, weak but clearly positive IF reactions with viable *P. knowlesi* sporozoites were detected in 2 out of 30 sera from the Malaysian group (Table 6).

Table 5. Comparison of antibody titres against *P. falciparum* sporozoites and IRBC in serum of West African patients and sporozoite-immunized volunteers

Origin of sera	Immunofluorescent titres	
	P. falciparum sporozoites viable	*P. falciparum* IRBC
West African patients:[a]		
G-3	pos.[b]	1 4096
G-15	pos	1.4096
G-36	pos	1 256
G-38	pos	1 1024
P. falciparum immunized volunteer[c]	1:4096	1 16
Controls		
P. vivax immunized volunteer	neg.	1·16
Normal human donor	neg	neg

[a] MRC Clinic, Gambia, West Africa
[b] Positive at 1.16, not titrated further
[c] Immunized with Burma (Thau) strain sporozoites

We attempted to enhance the titre of the weak IF reactions obtained with viable sporozoites by using a double-sandwich technique. Although this procedure increased the intensity of the fluorescence, the titre was not increased, i.e., these reactions were not positive beyond a 1:4 dilution of the sera. When sporozoites fixed with glutaraldehyde were used as antigen, however, the titre of the antisporozoite antibodies was 1:64 while the controls (Table 6) remained negative.

We were interested, therefore, in determining whether the use of glutaraldehyde-fixed sporozoites would also enhance the detection of anti-sporozoite antibodies in the sera of humans from endemic areas. We obtained sera from an area endemic for *P. malariae* and *P. vivax* (*27*)[b] and sporozoites from *P. vivax*-infected *A. stephensi*.

Three human sera were tested. All 3 had high anti-IRBC titres (1:1024–1:4096) but were CSP negative

[a] This serum was kindly provided by Dr Vincent McCarthy, University of Maryland School of Medicine.

[b] These sera were kindly provided by Dr A. Sulzer, Center for Disease Control, Atlanta, GA.

against viable sporozoites. These undiluted sera gave weakly positive IF reactions with viable sporozoites and 1:4–1:16 titres with glutaraldehyde-fixed sporozoites. In addition, the titre of a serum sample from a volunteer immunized with *P. vivax* (*24*) was increased from 1:256 with viable *P. vivax* sporozoites to 1:4096 with glutaraldehyde-fixed sporozoites. Control sera did not react with these fixed *P. vivax* sporozoites.

These results indicate that antisporozoite antibodies against *P. falciparum*, *P. vivax*, and *P. knowlesi* could be detected in sera from naturally infected individuals living in malaria-endemic regions. Further studies, planned for the near future, may provide information regarding the incidence and level of antisporozoite antibodies in endemic areas, as well as the persistence of these antibodies in relation to the transmission season. It is certainly important to determine whether ongoing malarial transmission produces an antisporozoite immune response; this could reinforce and prolong antisporozoite immunity, which in the future might be induced by immunoprophylactic measures.

Further characterization and isolation of the surface antigens of sporozoites would represent an important step toward understanding plasmodial-host relationships. Surface antigens are likely to play a fundamental role in plasmodial penetration into vertebrate host cells, which represents the first phase of all malarial infections. Furthermore, it is plausible that protective antigens are localized on the sporozoite surface. Clarification of the role of surface antigens in host immunity may therefore facilitate the development of a successful malarial vaccine.

Table 6. Comparison of antibody titres against *P. knowlesi* sporozoites and IRBC in feral *Macaca fascicularis* (= *M. irus*) and immunized rhesus monkeys

Origin of sera [a]	*P knowlesi* sporozoites			*P knowlesi* IRBC
	Viable		Fixed [b]	
	CSP	IF	IF	IF
M-1	neg.	neg	neg	1:4
M-2	neg	1:4W	1:64	1:16
M-3	neg.	1 4W	1 64	1 16
M-4	neg	neg	neg	neg.
M-5	neg.	neg	neg	1.16
M-6	neg	neg.	neg.	neg
M-7 – M-30	neg.	neg	neg.	N.D.
Rhesus monkeys immunized with *P. knowlesi* sporozoites	1:4	1.1024	1 4096	N.D.
Controls				
Rhesus monkeys immunized with *P. knowlesi* IRBC	neg	neg.	neg	1 256
Normal rhesus monkeys	neg.	neg	neg	neg.

[a] M numbers represent *Macaca fascicularis* monkeys from Malaysia

[b] 1 g/litre (0.1%) gluteraldehyde.

ACKNOWLEDGEMENTS

We are particularly grateful to Dr R. S. Bray (MRC, The Gambia) and Dr Richard Carter (Laboratory of Parasitic Diseases, NIH). In addition, we should like to thank Dr Hannah Shear for reading the manuscript.

This research was made possible by grants from the Agency for International Development, the National Institutes of Health, and the World Health Organization.

RÉSUMÉ

CARACTÉRISATION DES ANTIGÈNES DE SURFACE DU SPOROZOÏTE PAR L'IMMUNOFLUORESCENCE INDIRECTE· DÉTECTION D'ANTICORPS ANTIPARASITAIRES SPÉCIFIQUES DU STADE ET DE L'ESPÈCE

Le rôle que jouent peut-être dans l'apparition spontanée de l'immunité les antigènes parasitaires au stade du sporozoïte n'a pu encore être que partiellement exploré, faute d'une technique sérologique applicable dans les études sur le terrain. Pour localiser sur la membrane du sporozoïte tout antigène spécifique de ce stade, les auteurs ont appliqué la technique d'immunofluorescence indirecte (IF). Leurs expériences avaient pour but d'examiner s'il était possible d'employer cette technique pour les études séroépidémiologiques visant à déceler la présence d'une réponse dirigée contre les sporozoïtes chez les individus vivant dans des zones d'endémie.

Pour évaluer la spécificité et la sensibilité des épreuves IF, celles-ci ont été pratiquées d'abord avec des sérums de rongeurs et de sujets simiens ou humains immunisés contre les sporozoïtes ou contre les stades sanguins du parasite. Une réaction de fluorescence n'est apparue chez les sporozoïtes que lorsqu'ils avaient été incubés avec des immunsérums dirigés contre les sporozoïtes de la même espèce de parasite du paludisme. On n'a pas décelé de réaction croisée avec des antisérums dirigés contre des sporozoïtes d'espèces hétérologues. Pour la détection d'anticorps spécifiques du stade, c'est-à-dire anti-sporozoïtes, l'incubation des immunsérums avec des sporozoïtes viables ou fixés au glutaraldé-

hyde était nécessaire. En présence d'antisérums dirigés contre les stades sanguins, la réaction exige la rupture préalable des sporozoïtes par dessèchement à l'air et congélation. La spécificité des anticorps anti-sporozoïtes quant à l'espèce et quant au stade, mise en évidence par l'immunofluorescence indirecte, corrobore les résultats obtenus avec l'épreuve de précipitation circumsporozoïtaire (CSP). Mais les épreuves IF se sont montrées plus sensibles puisqu'elles ont permis de déceler des titres d'anticorps 5 à 10 fois supérieurs à ceux détectés par l'épreuve CSP.

Dans une série d'expériences préliminaires, on a appliqué les épreuves IF en vue de déterminer si, dans les cas d'infection naturelle chez l'homme, les sérums des individus atteints contenaient des anticorps anti-sporozoïtes décelables. Les sujets en petit nombre sur lesquels ont été prélevés les sérums habitaient des régions impaludées d'Afrique ou d'Amérique du Sud. Des prélèvements ont également été opérés sur quelques singes de l'espèce *Macaca fascicularis*. Selon les premiers résultats, la présence d'anticorps contre *Plasmodium falciparum*, *P. vivax* et *P. knowlesi*, spécifiques du stade et de l'espèce, peuvent être décelés par l'épreuve IF dans les sérums d'habitants des zones d'endémie naturellement infectés. Les titres d'anticorps anti-sporozoïtes décelés étaient plus élevés lorsque l'antigène utilisé était constitué par des parasites fixés au glutaraldéhyde. L'emploi de ce matériau a facilité la collecte et le stockage de l'antigène en vue d'études plus vastes sur le terrain.

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