DETERMINANTS ON SURFACE PROTEINS OF *PLASMODIUM KNOWLESI* MEROZOITES COMMON TO *PLASMODIUM FALCIPARUM* SCHIZONTS

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Past observations on serologic cross-reactions between malarias of different species indicated that malaria parasites have common antigenic determinants (interspecies antigens) (1, 2). However, the molecular nature and subcellular localization of interspecies antigens have never been determined. Such an analysis is complicated by the fact that the malaria parasite has morphologically distinct stages in its life cycle (i.e., sporozoites, asexual erythrocytic parasites, and gametes). Furthermore, antigens of a particular stage may be on the surface or located internally. Even asexual erythrocytic parasites, the focus of this study, have developmental stages. On invasion of an erythrocyte, the merozoite rapidly transforms into a ring form. Developmental stages include the trophozoite and the schizont, the latter stage being a dividing parasite that contains, at full maturation, individual merozoites. When the infected erythrocyte ruptures, the released merozoites have a short extracellular life in the plasma before invading other erythrocytes. New antigens are expressed as the parasite develops from its ring form to the schizont stage (3). Parasite antigens also appear on the erythrocyte membrane as the intraerythrocytic parasite matures (4). Extracellular merozoites are antigenically similar to schizont-infected erythrocytes (3).

Recently, Schmidt-Ullrich et al. (5) demonstrated that malaria antigens in the infected erythrocyte membrane from the Malaysian and Philippine strains of *Plasmodium knowlesi* had common antigenic determinants. Importantly, these surface determinants were the main antigens recognized by antibodies in sera from monkeys rendered immune by prior *P. knowlesi* infection. These observations raised the possibility that surface antigens on extracellular merozoites, as well as membranes of infected erythrocytes, even from different species of malaria may have determinants in common. In the present study we have determined the cross-reactivity between surface antigens on *P. knowlesi* merozoites and *P. falciparum* schizont-infected erythrocytes. Sera from adults who live in the Gambia, an area of West Africa hyperendemic for *P. falciparum* malaria, bind to the surface of intact *P. knowlesi* merozoites, although Gambian adults have never been exposed to this Asian malaria of monkeys. In addition, antibodies in the sera form complexes with the Triton X-100 (Rohm and Haas, Philadelphia, Pa.) -solubilized surface determinants from *P. knowlesi* merozoites. Triton extracts from *P. falciparum* schizonts inhibited the immune reaction between

these human sera and the *P. knowlesi* merozoite extracts. Thus multiple determinants on the surface of *P. knowlesi* merozoites have regions antigenically similar to those on *P. falciparum*. The identification of surface proteins common to asexual parasites of different species may allow for a classification system for these proteins as to their structure, function, and role in induction of protective immunity.

Materials and Methods

Isolation of P. knowlesi Merozoites. Viable merozoites were obtained from short-term culture as described in detail elsewhere (6). Briefly, venous blood was drawn into a heparinized syringe from a rhesus monkey infected with the Malaysian strain of *P. knowlesi*. At the time of study the parasitemia was between 10 and 35% and the majority of infected erythrocytes contained mature schizonts. Schizont-infected erythrocytes were separated from the majority of lymphocytes, platelets, uninfected erythrocytes, and plasma; suspended in culture medium (RPMI-1640 [Grand Island Biological Co., Grand Island, N. Y.] with 15 mM Hepes, 34.5 mM $NaHCO_3$, and 10 μg/ml gentamycin); and placed in a culture chamber at 37°C. The atmosphere above the medium contained 5% CO_2 in air. The chamber floor was covered by a polycarbonate membrane with 1.5-μm holes through which merozoites, but not schizont-infected erythrocytes, could pass. Soon after release from schizont-infected erythrocytes, merozoites were collected for study.

Labeling of Merozoite Surface Proteins with ^{125}I. 10 ml of culture-chamber effluent that contained merozoites was diluted with 30 ml of a balanced salt solution (80 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 10 mM glucose, 20 mM Na_2HPO_4, 2 mM NaH_2PO_4, pH 7.4) and centrifuged for 5 min at 10,000 *g*. The merozoite pellet was gently resuspended in 1 ml of balanced salt solution (~5×10^8 merozoites/ml). Radioiodination was performed as described elsewhere (7). To the merozoite suspension was added 50 μl of lactoperoxidase (1 mg/ml), 30–50 μl of carrier-free [^{125}I]NaI (~17 Ci/mg) (New England Nuclear, Boston, Mass.), followed by 50 μl of 0.2 mM H_2O_2. 1 min after adding H_2O_2, the mixture was diluted with 50 ml of balanced salt solution and washed three times to remove the free ^{125}I-Na that was not linked to protein. Evidence that the lactoperoxidase catalyzed iodination was on surface proteins will be published in detail elsewhere (J. G. Johnson, N. Epstein, and L. H. Miller. Unpublished data.). Mild trypsin treatment of intact, lactoperoxidase ^{125}I-labeled merozoites preferentially cleaved labeled proteins. Internal proteins labeled by other methods were resistant to enzyme treatment.

Extraction of ^{125}I-labeled Merozoites. The pellet of washed merozoites was extracted with 1% Triton X-100 with 1 mM Hepes, pH 8, and 0.2 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK)[1] for 60 min at 4°C. The mixture was then centrifuged at 140,000 *g* for 60 min in an Airfuge (Beckman Instruments, Inc., Fullerton, Calif.), and the soluble extract was stored at −70°C. Before being used in analyses that involved fixed *Staphylococcus aureus* (Cowan I) cells, (Pansorbin, Calbiochem-Behring Corp., American-Hoechst Corp, San Diego, Calif.), the labeled soluble extract was absorbed with fixed *S. aureus* cells in the absence of antibody, a step that greatly reduced the background antibody-independent binding of Triton extract to fixed *S. aureus* cells. 50 μl of the Triton extract was mixed with a wet pellet of 0.2-g-fixed *S. aureus* cells. After 1 h at room temperature the mixture was centrifuged at 12,000 *g* for 4 min, and the supernate was used for immunochemical and electrophoretic analyses.

Preparation of Extracts from P. falciparum and P. knowlesi Schizonts for Inhibition Studies. Ideally, one would have liked to compare antigens of *P. knowlesi* and *P. falciparum* merozoites. However, for technical reasons, viable merozoites can not be obtained from *P. falciparum*; therefore, schizont-infected erythrocytes were used. The *P. falciparum* schizonts were obtained from culture, and *P. knowlesi* schizonts were obtained from infected rhesus monkeys.

The Malaysian Camp strain of *P. falciparum* was grown continuously in stock cultures with a modification of the methods of Trager and Jensen (8) and Haynes et al. (9). Parasitized erythrocytes from a stock culture were mixed with freshly washed human type O^+ erythrocytes

[1] *Abbreviations used in this paper:* IFA, indirect fluorescent antibody; SDS, sodium dodecyl sulfate; TLCK, *N*-α-*p*-tosyl-L-lysine chloromethyl ketone.

so that 1% of the erythrocytes were infected, and then the culture was synchronized by sorbitol lysis of mature parasites (modified from Lambros and Vanderberg, [10]). For sorbitol lysis, the culture was centrifuged at 350 *g* for 5 min at room temperature, the supernatant fluid was aspirated, and the pellet was resuspended in nine packed cell volumes of 5% wt/vol sorbitol (Calbiochem-Behring Corp., American Hoechst Corp.) in distilled water for 5 min at 37°C. The cell suspension was vortexed vigorously for 15 s, centrifuged again, and the pellet resuspended to 6% vol/vol in culture medium, which consisted of RPMI-1640 made from powder with a 5% excess of sterile distilled water, with 25 mM Hepes, 32 mM $NaHCO_3$, and 10% heat-inactivated human serum. The suspension (15 ml) was recultured in a 75-cm^2 flask (Corning Glass Works, Science Products Div., Corning, N. Y.), which was flushed with a gas mixture that consisted of 5% O_2, 5% CO_2, and 90% N_2, sealed, and incubated at 37°C. When reinvasion occurred, and new rings began to appear again in the culture, the culture was again treated with 5% sorbitol to destroy trophozoites and schizonts, thus increasing the synchrony. About 40 h after the second sorbitol treatment, when most of the parasites were schizonts (and 1% of the erythrocytes contained parasites), the erythrocytes were washed twice in medium without serum. The packed erythrocytes were mixed with an equal volume of 0.2% saponin (Fisher Scientific Co., Pittsburgh, Pa.) in 0.15 M NaCl and 0.2 mM TLCK and continuously agitated for 30 min. The lysed parasitized erythrocytes were washed twice in RPMI-1640 with 15 mM Hepes and 0.2 mM TLCK. The cells were centrifuged at 850 *g* for 10 min for each wash. The final pellet contained lysed parasitized erythrocytes and an equal number of unlysed normal erythrocytes. The pellets were extracted in 1% Triton as described above and stored at −70°C.

Blood from a rhesus monkey that contained predominantly mature *P. knowlesi* schizonts was handled in a similar manner to the preparation of *P. falciparum* schizonts to obtain a Triton extract of *P. knowlesi* schizonts.

As controls, extracts were also made from erythrocyte ghosts of a normal, uninfected man and monkey. Rhesus and human erythrocytes were washed as described above, lysed in 0.1% saponin that was washed by centrifugation at 48,000 *g* for 10 min, and extracted in 1% Triton. The ghosts in this extraction were hemoglobin free. Rhesus hemoglobin was prepared from washed rhesus erythrocytes by freeze-thawing the cells three times. The ghosts were separated from the hemoglobin by centrifugation at 27,000 *g* for 15 min.

The concentration of protein in each extract was determined by a modification of the Lowry method (11). The protein concentration for each antigen used in competition studies was as follows: *P. falciparum*, 15 mg/ml; *P. knowlesi*, 36 mg/ml; human erythrocyte ghosts, 1.4 mg/ml; rhesus erythrocyte ghosts, 1.5 mg/ml. Before Triton extraction, *P. knowlesi* antigen consisted of parasitized erythrocytes (1.8×10^9/ml), uninfected erythrocytes (1.1×10^9/ml), and leukocytes (0.3×10^9/ml). Because of incomplete saponin lysis, the Triton extract contained 28 mg/ml hemoglobin. The limited supply of *P. falciparum* antigen did not permit similar analysis. It should be noted, however, that the *P. falciparum* antigen would contain only rare leukocytes, because the erythrocytes were separated from the leukocytes before the erythrocytes were used in culture.

Sera. 15 sera from adult Gambians were reacted with ^{125}I-labeled *P. knowlesi* merozoites. Five that bound the most antigen and one (G-21) that bound the least were chosen for further study. Sera from adults from the United States who were not immune to malaria were the controls.

Immunochemical Studies. Extracts from ^{125}I-labeled merozoites were mixed with various dilutions of Gambian sera or normal sera and incubated at 4°C for 18 h. 100 μl of fixed *S. aureus* cells that had been washed once with 0.15 M NaCl that contained 10 mM Tris and 0.25% Triton was added to the antigen-antibody mixture. After 30 s, 1.5 ml of the NaCl-Tris-Triton solution was added, and the suspension was centrifuged at 1,100 *g* for 10 min. The pellet was washed two more times. The radioactivity in the pellet and supernate from the washes was determined in a gamma counter, and the percentage of the total radioactivity recovered in the pellet was calculated.

Antigen Competition Studies. Extracts of *P. falciparum* schizonts, *P. knowlesi* schizonts, rhesus erythrocyte ghosts, rhesus hemoglobin, or human erythrocytes were tested for the ability to inhibit binding of extracts of ^{125}I-labeled *P. knowlesi* merozoites with antibody in Gambian serum (G16). 10 μl of ^{125}I-labeled merozoites (~7,500 cpm) was mixed with 10 μl of various

dilutions of inhibiting antigen. The inhibiting antigen was diluted in RPMI-1640 with Hepes. Then, 10 μl of a Gambian serum (G16) diluted 1:100 in 0.15 M NaCl, 10 mM Tris (pH 7.4), and 5% bovine serum albumin (30% bactobovine albumin) was added, and the mixture was incubated at 4°C for 16 h. *S. aureus* cells were added, and the pellet was obtained as described above.

The amount of radioactive merozoite protein bound in the presence of serum from a nonimmune United States donor was measured after absorption with *S. aureus* cells. This background (counts bound with nonimmune serum) was subtracted from counts obtained with Gambian serum in the presence or absence of inhibitor. The percentage of inhibition was determined for each antigen as follows:

$$\% \text{ inhibition} = 100 - \frac{\text{cpm (G16 with inhibitor)} - \text{cpm (background)}}{\text{cpm (G16 without inhibitor)} - \text{cpm (background)}} \times 100.$$

Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gel Electrophoresis. Pellets of *S. aureus* cells that contained bound immune complexes prepared as described above were washed twice with phosphate-buffered saline that contained 0.25% Triton X-100, then were eluted with 100 μl of electrophoresis sample buffer at 70°C for 30 min. The sample buffer contained 2% SDS, 2% 2-mercaptoethanol, 62.5 mM Tris-HCl (pH 6.8), 10% glycerol, and 0.001% Bromophenol Blue. *S. aureus* cells were removed by centrifugation, and the supernates were electrophoresed on 8–12% polyacrylamide gradient slab gels as described by Laemmli (12). Gels were fixed in 10% TCA, stained with Coomassie Blue, dried, and autoradiographed. Standard molecular weight markers were used: myosin (200,000), β-galactosidase (130,000), phosphorylase (94,000), bovine serum albumin (68,000), catalase (57,500), aldolase (40,000), carbonic anhydrase (30,000), and soybean trypsin inhibitor (21,000).

Inhibition of Invasion. To test whether the Gambian sera would block invasion, 15 ml of culture-chamber effluent that contained merozoites was centrifuged at 10,000 *g* for 5 min at room temperature and resuspended in 1.2 ml of culture medium. 100 μl of the suspension was added to 10 μl of heat-inactivated serum and incubated for 10 min at room temperature. Then, 100 μl of rhesus erythrocytes (100,000/mm^3 in Medium 199 with 10% inactivated fetal calf serum) was added to each sample. The suspension was mixed for 30 min at 37°C to allow for invasion, and the number of ring-infected erythrocytes was enumerated from a Giemsa stain thin-blood film.

Immunofluorescent Studies. Test for antibodies against exposed determinants on *P. knowlesi* merozoites was as follows. Merozoites in the culture chamber effluent were centrifuged at 12,000 *g* for 2 min. The pellet was resuspended in a 1:20 dilution of various sera for 10 min at room temperature. The merozoites were washed two times in culture medium and resuspended in fluorescent-tagged goat anti-human Ig for 10 min at room temperature. The merozoites were observed for fluorescence.

Results

Adults from Gambia, West Africa, are highly immune to *P. falciparum* from repeated exposure to the parasite throughout their lives.

Gambian sera (G5, 6, 15, 16, 40) had high indirect fluorescent antibody (IFA) titers against *P. falciparum* schizonts (1:1,280–1:20,000) (Table I), which indicates that they were from highly immune individuals. Another Gambian serum (G21) that had an IFA titer against *P. falciparum* of only 1:80 was also studied. According to indirect immunofluorescence, antibodies in the five highly immune sera (diluted 1:20) bound to the surface of intact, viable *P. knowlesi* merozoites (Table I). Antibodies in sera from nonimmune individuals and G21 did not bind to merozoites. None of the Gambian sera, diluted 1:10 and preincubated with *P. knowlesi* merozoites, reduced the parasite's ability to invade rhesus erythrocytes (Table I). As a comparison, serum from a monkey specifically immunized against *P. knowlesi* merozoites consistently caused a 5- to 50-fold decrease in invasion.

TABLE I

Antimalarial Activity of Sera from Six Adult Gambians (G5 to 40) and from a United States Nonimmune Control

Subjects	Age	IFA* *P. falciparum*	IFA* *P. knowlesi*	IFA‡ merozoite suspension	^{125}I-merozoite§ antigen	Percent invasion	
						Exp. 1	Exp. 2
	yr				*cpm bound*	%	
G5	30	1,280	320	+	7,400	5.5	10.9
G6	14	5,120	320	+	6,300	9.6	12.1
G15	20	5,120	1,280	+	4,400	9.8	12.3
G16	50	1,280	1,280	+	9,200	6.3	7.1
G40	73	20,000	320	+	4,100	10.0	10.6
G21	48	80	80	−	500	2.3	5.8
Control	44		Negative	−	300	5.0	7.7

* IFA reciprocal titers against *P. falciparum* schizonts or *P. knowlesi* merozoites dried on slides.

‡ Viable *P. knowlesi* merozoites were incubated in suspension with a 1:20 dilution of sera at room temperature for 10 min and then reacted with fluorescent-tagged goat anti-human Ig. +, surface fluorescence present; −, no fluorescence.

§ Triton extracts of ^{125}I-lactoperoxidase-labeled merozoites (46,000 cpm) were incubated with a 1:3 dilution of sera. The immune complexes were bound to *S. aureus* cells, and the bound counts were determined.

‖ Percent rhesus erythrocytes invaded after incubation of merozoites with a 1:10 dilution of sera for 10 min.

To determine whether cross-reactivity exists between human malaria and *P. knowlesi*, we incubated sera from five adult Gambians with Triton extracts of proteins from ^{125}I-surface-labeled *P. knowlesi* merozoites and reacted the immune complexes with fixed *S. aureus* cells. The complexed proteins were eluted off the *S. aureus* and analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 1). All major polypeptide bands seen in the original Triton extract (slot 1) reacted with the Gambian immune sera (slots 4–8). Sera from a nonimmune United States control (slot 3) and from an apparently nonimmune Gambian (G21, slot 9) reacted to a small extent with a 54,000-dalton protein. This represents background antibody-independent binding to *S. aureus* cells, as the same amount of binding occurred when serum was replaced by buffer.

High titer antibodies to asexual stages of *P. falciparum* in Gambian sera as determined by indirect immunofluorescence were the likely source of cross-reacting antibodies to *P. knowlesi* merozoites. We tested this hypothesis by competition for antibody in Gambian serum (G16) between Triton-extracted *P. falciparum* schizonts and antigen derived from the surface-labeled *P. knowlesi* merozoites. In preliminary experiments, it was found that, at a dilution of 1:300, the amount of radioactivity bound began to fall. This dilution was therefore used for competition studies. As shown in Fig. 2 A, *P. falciparum* antigen inhibited the reaction between the serum and extracts of ^{125}I-labeled *P. knowlesi* merozoites. To determine if the competing antigen (extract of *P. falciparum*) was selective for only some proteins in the *P. knowlesi* merozoite extract or inhibited the reaction of all proteins, the following procedure was employed. The labeled protein bound to *S. aureus* cells at various dilutions of inhibiting antigens was eluted in 2% SDS and electrophoresed on polyacrylamide gels. The presence of *P. falciparum* antigen inhibited antibody complexes with all proteins (Fig. 3). As expected, extracts of *P. knowlesi* schizonts also inhibited the immune reaction between Gambian serum and ^{125}I-labeled *P. knowlesi* merozoites (Figs. 2 B and 3).

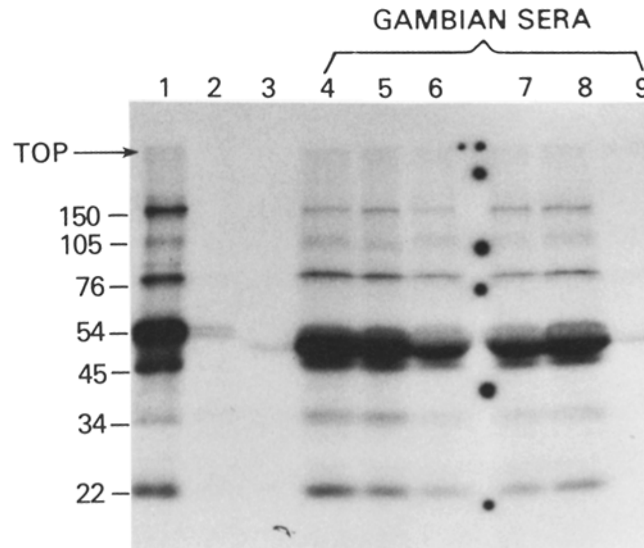

FIG. 1. SDS-polyacrylamide gel electrophoresis of immune complexes. The autoradiogram is shown. Slot 1 contains the original Triton extract of ^{125}I-surface-labeled *P. knowlesi* merozoites. Samples in slots 3–9 were prepared with the following sera at a final dilution of 1:3, in order: nonimmune United States donor, G16, G5, G40, G15, G6, and G21. Serum was replaced by buffer in slot 2. Molecular weights (in thousands) appear at the left. Single black dots between slots 6 and 7 mark standard polypeptides: soybean trypsin inhibitor (lowest dot) (21,000), aldolase (40,000), bovine serum albumin (68,000), phosphorylase (94,000), and myosin (200,000). The ^{125}I-labeled bands around 54,000 are pushed further down the gel in slots 3–9 as a result the large amount of IgG heavy-chain polypeptide that travels just behind the 54,000 band.

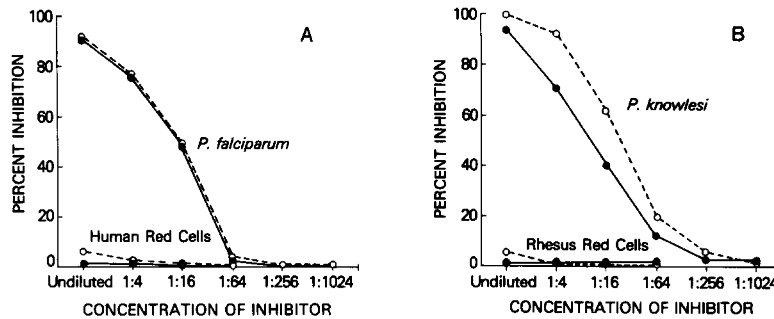

FIG. 2. Inhibition by Triton of *P. falciparum* (A) and *P. knowlesi* (B) schizonts of immune complexes between Gambian serum (G16) and Triton extracts of ^{125}I-surface-labeled *P. knowlesi* merozoites. The immune complexes were bound to *S. aureus* cells, and the washed pellets were counted. Human and rhesus erythrocyte ghosts were tested as controls for normal erythrocyte components.

Because *P. falciparum* and *P. knowlesi* antigens contained normal erythrocyte components, these erythrocyte components were also tested for inhibition. Neither human erythrocytes (Fig. 2 A), rhesus erythrocytes (Fig. 2 B), nor hemoglobin (6.25–50 mg/ml [data not shown]) inhibited the immune reaction between Gambian serum and ^{125}I-labeled *P. knowlesi* merozoites.

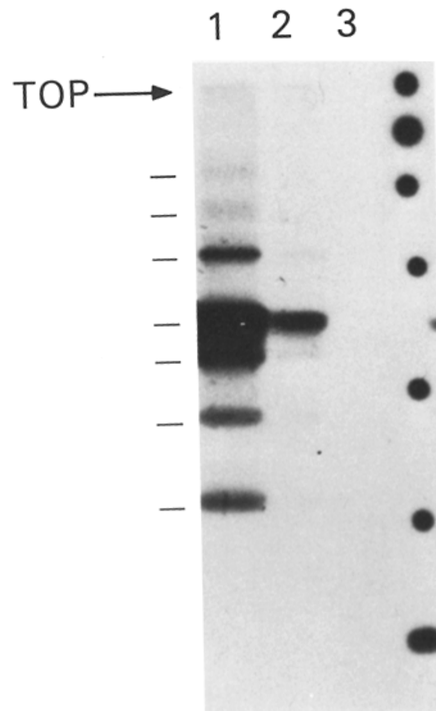

FIG. 3. SDS-polyacrylamide gel electrophoresis of immune complexes between Triton extract of ^{125}I-surface-labeled *P. knowlesi* merozoites and serum G16 (slot 1) and of inhibition of these complexes with Triton extracts of *P. falciparum* (slot 2) and *P. knowlesi* (slot 3). Black dots mark standards: dye front (lowest dot), soybean trypsin inhibitor (21,000), aldolase (40,000), bovine serum albumin (68,000), β-galactosidase (130,000), and myosin (200,000).

Discussion

Although it has been observed previously that antibodies to one species of malaria will cross-react with other species (1, 2), the parasite antigens (i.e., surface or internal antigens) to which these cross-reacting antibodies bind have not been defined. Sera from Gambians who are highly immune to *P. falciparum* malaria as a result of repeated natural infections are an ideal reagent to use in a search for cross-reacting antigens. Indeed, it was observed in this study that antibodies in these sera recognized proteins on the surface of *P. knowlesi* merozoites. The evidence was as follows. First, indirect immunofluorescent studies demonstrated that the antibodies bound to the surface of intact *P. knowlesi* merozoites. Second, the antibodies formed immune complexes with Triton extracts of ^{125}I-labeled surface proteins on *P. knowlesi* merozoites. Because Gambians are not exposed to *P. knowlesi*, the antibodies must be directed against antigens associated with another parasite, probably *P. falciparum*. The fact that antigens extracted from *P. falciparum* schizonts inhibited the reaction between Gambian serum and *P. knowlesi* merozoites indicates that these two plasmodial species have common antigens. A direct comparison of the antigenic composition between *P. knowlesi* and *P. falciparum* merozoites has not been possible because to date only *P. knowlesi* merozoites can be isolated in a viable, invasive state. The stage before release of merozoites, schizont-infected erythrocytes, appears to share all major antigens with merozoites (3) including merozoite surface antigens (Fig. 3).

Although the targets of the immune response in vivo are unknown, immunity would presumably be directed against malaria determinants on membranes of infected erythrocytes or the surface of merozoites. In this study and (R. Schmidt-Ullrich, L. H. Miller, D. F. H. Wallach. Manuscript in preparation.), we have shown that malaria antigens on the surface of merozoites and of infected erythrocytes have common determinants between a monkey malaria of Asia, *P. knowlesi*, and malignant *P. falciparum* malaria of man. Despite the cross-reactivity for surface determinants between *P. knowlesi* and *P. falciparum*, it is not known if protective immunity to one of these malarias would lead to protection against the other. In general, however, protective immunity against the asexual infection in primate malarias is species specific (13–15). There is no cross-protection between *P. vivax* and *P. falciparum* in man (13) or owl monkeys (14). Rhesus monkeys that were highly immune to *P. knowlesi* as a result of chronic infection with this species were still fully susceptible to infection with *P. cynomolgi* or *P. coatneyi* (15). Possible cross-protection between certain primate malarias has been noted (13, 15). For example, prior infection of man with *P. ovale* may reduce the severity of a subsequent infection with *P. falciparum*. As mentioned above, the cross-protection between *P. falciparum* and *P. knowlesi* has yet to be tested in vivo. However, in vitro studies on the effect of serum on merozoite invasion of erythrocytes may give some indication of the immune state of the host (16). In the present study, sera from Gambian adults who were highly immune to *P. falciparum* did not reduce erythrocyte invasion by *P. knowlesi* merozoites, although antibody in the sera bound to the surface of merozoites. If cross-protection does not exist, as seems likely from the above discussion, then determinants critical for protective immunity may not cross-react. Alternatively, the cross-reactive determinants may be on unexposed portions of surface components, whereas the exposed portions that are important in induction of protective immunity do not cross-react. It is also possible that the antibody must be of the proper class, specificity, or affinity to afford protection.

Summary

In this report and (R. Schmidt-Ullrich, L. H. Miller, and D. F. H. Wallach. Manuscript in preparation.), we have demonstrated that malaria proteins on the surface of merozoites and infected erythrocytes cross-react between at least two primate malarias, *Plasmodium knowlesi* and *P. falciparum*. Sera from five Gambian adults who were highly immune to *P. falciparum* were used as a reagent to study the cross-reactivity between *P. falciparum* schizonts and surface proteins on *P. knowlesi* merozoites. Although the sera bound to the surface of viable, intact *P. knowlesi* merozoites, the sera did not block invasion of rhesus erythrocytes. ^{125}I-lactoperoxidase-labeled surface proteins on merozoites formed complexes with the antibody. All major protein bands seen in the electrophoresis of the original Triton extract were bound by the immune sera. Because Gambians have never been exposed to *P. knowlesi* malaria, the antibodies that reacted with *P. knowlesi* merozoites must be directed against antigens of another parasite such as *P. falciparum*. We tested this hypothesis by competition for antibody in a Gambian serum between Triton-extracted antigens from *P. falciparum* schizont-infected erythrocytes and from surface-labeled *P. knowlesi* merozoites. *P. falciparum* inhibited the reaction, thus indicating cross-reaction between antigens in *P. falciparum* schizonts and *P. knowlesi* merozoites.

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