Characterization and Localization of Plasmodium falciparum Surface Antigens on Infected Erythrocytes From West African Patients


The malaria-induced surface antigens on Plasmodium falciparum-infected erythrocytes from West African patients were characterized by agglutination of infected cells by human sera, surface immunofluorescence of live infected cells, inhibition of cytoadherence to C32 melanoma cells by human sera, immunoelectron microscopy (immunoEM), and immunoprecipitation. In a nonimmune individual, serum antibody reactivity to surface antigens of infected cells was acquired during convalescence, as tested by all five methods, and was generally parasite isolate-specific. By contrast, adult hyperimmune West African sera reacted with many isolates, including isolates from geographically distinct regions. A quantitative correlation was established between agglutination and surface immunofluorescence assay titers, and between surface immunofluorescence assay and immunoEM reactivity, suggesting that a single antigen or a set of coexpressed antigens is being detected. Surface iodination of infected cells identified trypsin-sensitive high M, antigens in the sodium dodecyl sulfate extract. All sera tested that agglutinated infected cells also immunoprecipitated these antigens. The same surface antigens were immunoprecipitated by the homologous convalescent serum as by adult sera. By immunoEM these antigens were localized exclusively at the knob-like protrusions of infected cells, where they may participate in adherence to vascular endothelium.

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An important factor in Plasmodium falciparum malaria is the ability of the parasite to sequester in selected tissues of the host through the adherence of the mature infected erythrocytes to endothelial cells lining blood capillaries. Studies in Aotus and Saimiri monkeys on the virulence of knob-bearing (K+) parasites, which sequester, and knobless (K-) parasites, which do not, showed that infected cell adherence is important for parasite survival in vivo. Adherence is probably also an important factor in malaria pathology, especially in cerebral malaria. Identification of the malaria receptor for endothelial cells is therefore important both for the design of an effective vaccine and for potential development of receptor analogues that could reverse adherence in acute malaria.

A major criterion for identification of the adherence receptor is that it must be exposed on the surface of the infected red blood cell (RBC) to mediate binding to endothelial cells. While approximately 10 different P. falciparum or modified host proteins associated with the infected erythrocyte membrane have been described, only some of these have been shown to be exposed on the outside surface of the membrane. One such candidate for the malarial cytoadherence receptor, P. falciparum-infected erythrocyte membrane protein 1 (PIEMP1), has been well characterized in Aotus-adapted and culture-adapted P. falciparum strains. PIEMP1 is exposed on the surface of infected RBC and can be detected by agglutination or surface immunofluorescence with variant-specific antisera. PIEMP1 is a size-variant 200- to 300-Kd molecule, readily surface-labeled with [125I] on intact infected erythrocytes, and insoluble in Triton X-100 (TX100), presumably due to its association with cytoskeletal elements. The properties of PIEMP1 correlate with those expected of the parasite cytoadherence molecule, because (1) cytoadherence in a number of in vitro and in vivo models correlates with the expression of PIEMP1; (2) both in vitro cytoadherence and cell surface PIEMP1 are sensitive to low levels of trypsin; and (3) the ability of antisera from Aotus monkeys to block or reverse cytoadherence in vitro correlates with their ability to react with PIEMP1 in agglutination or immunoprecipitation assays. However, there is as yet no direct proof for the function of PIEMP1. Furthermore, even if PIEMP1 is important for cytoadherence, there may be additional surface antigens that are involved. Several potential host receptors have been identified, and these may bind to different parasite receptors. Furthermore, a single clone of P. falciparum is capable of expressing multiple PIEMP1-like proteins.

Using serologic techniques such as agglutination and immunofluorescence, several studies have demonstrated the presence of variable surface antigens on infected RBC taken directly from West African patients. While children acquire isolate-specific agglutinating antibodies during convalescence from a particular episode of malaria, immune adults have antibodies capable of reacting with many isolates, perhaps through recognition of conserved
determinants. Acquisition of agglutinating antibodies was the only immune factor, among a number of antibody-dependent phenomena tested, to show a consistent protective effect against clinical malaria in a field study in The Gambia. A size-variant, high M, infected erythrocyte membrane antigen in West African isolates from human malaria patients has been demonstrated by surface iodination studies, but was not characterized serologically. Using specimens collected from acutely infected West African patients, we here present data comparing the surface properties of \textit{P.falciparum}-infected erythrocytes as detected by human sera from endemic areas of West Africa. The interaction of these serum antibodies with infected cells was studied by agglutination and surface immunofluorescence assays (IFA), inhibition of cytoadherence to C32 melanoma cells by human sera, immuno-electron microscopy (immunoEM), and the trypsin-sensitivity and immunoprecipitation of surface iodinated proteins of high M,. By these criteria, the wild human isolates of \textit{P.falciparum} appear to contain a PfEMP1 molecule similar to that seen in \textit{Aotus}- or culture-adapted isolates. We also show that the surface antigens are localized to the parasite-induced knob protrusions of the infected erythrocyte membrane.

**MATERIALS AND METHODS**

\textit{Parasite isolates and sera.} Primary isolates of \textit{P.falciparum}-infected blood were collected into heparin by venipuncture, from the dorsum of the hand of children or adults with malaria, at the outpatient clinic of the Medical Research Council (MRC) laboratories in Fajara, The Gambia or at the Pediatric Outpatients Section of Korle Bu Hospital, Accra, Ghana. Informed patient or parental consent was obtained before blood collection and all patients were treated with chloroquine immediately thereafter. Blood samples were washed three times in RPMI 1640 containing 20 mmol/L HEPES pH 7.2 and cryopreserved using glycerolyte (Fenwall/Travenol Labs, Deerfield, IL) as previously described. Thawed blood was cultured for 20 to 36 hours in vitro until the majority of parasites were mature trophozoites. \textit{P.falciparum} of the FVO strain was obtained from spleen-intact \textit{Aotus nancymai} monkeys held in Leticia, Colombia. Parasites were cryopreserved and cultured as described above.

Sera collected from 11 Gambian adults (denoted A7, A9, A11, 28, 142, 258, 261, 309, 758, 760, and TK669) were used individually or as a pool of several sera (denoted pool H). At the MRC Laboratories in Fajara, sera were also collected at the time of acute \textit{P.falciparum} infection (denoted 176AC and 434AC) or 3 weeks after chloroquine treatment and convalescence (denoted 176CO, 197CO, and 434CO). Sera were also collected from adults in rural Nigeria (denoted 1 and 7) and from adults at the following locations of rural Ghana: Akobima village (denoted AK1-13); Suhum (denoted SU-EH, SU-BO, etc) or from adults in Eastern Ghana as part of Burkitt’s lymphoma surveys (denoted BL-1-3). Control sera were obtained from two scientists (R.J.H. and T.H.) and are denoted NHS-1 and NHS-2. Monoclonal antibody (MoAb) 8B7.4 is a mouse αPfEMP2 (MESA) IgG1 (κ), which has been described previously.

**IFA.** The surface IFA on intact infected cells was performed as described. A tertiary IFA was used in all cases, using successively human serum, rabbit antihuman Fc\(_\gamma\) (anti-HuFc\(_\gamma\)), and fluorescein-conjugated goat antirabbit IgG. Antibodies were from Cappel Worthington Biochemicals (Malvern, PA) and were used at a 1/50 dilution in phosphate-buffered saline (PBS) containing 1\% bovine serum albumin (BSA). The titer of the human sera was taken to be the last dilution at which ≥25\% of \textit{P.falciparum}-infected RBC (PRBC) are IFA positive.

**Agglutination.** Agglutination assays were performed as described. Agglutination was quantitated by counting the number of PRBC in agglutinates in 40 consecutive fields. In Figs 1 and 2, the agglutination titer was taken to be the last dilution at which agglutinates of 3 or more infected erythrocytes, containing mature asexual parasites, were detected. In Figs 3 and 4, sera are scored as positive or negative based on a test at 1/5 dilution. In Fig 5A and B, micro-agglutinations are denoted positive (+) if strongly positive in three separate experiments at 1/5 dilution and detectable at greater dilutions, negative (−) if negative at 1/5 dilution in all three experiments, and weakly positive (±) if a weak positive reaction that titrated out by 1/10 dilution was detected in only some of the three experiments.

**Cytoadherence.** The assay was performed in triplicate as described. For inhibition studies using human sera, 1/5 dilutions of the human sera were preincubated with the PRBC at 0.5\% hematoctrit for 30 minutes at 37\°C before addition to the C32 cells.

**Iodination and immunoprecipitation.** Mature trophozoite PRBC were purified to ≥85\% parasitemia using Percoll/sorbitol gradients. Intact PRBC (1 to 5 \times 10\(^8\) cells) were labeled by the Na\(^{125}\)I-H\(_2\)O-lactoperoxidase method and sequentially extracted with 1\% wt/vol TX100 (Boehringer-Mannheim Biochemicals, Indianapolis, IN) and 2\% wt/vol sodium dodecyl sulfate (SDF). Extracts were immunoprecipitated with human sera and Protein A-Sepharose, and labeled antigens analyzed by SDS-polyacryla-

<table>
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*Fig 1. Agglutination reactivity of a human serum panel with isolate 434 and four other Gambian isolates.*
mide electrophoresis. Electrophoresis was performed on uniform 5% or 5% to 7.5% gradient gels to optimize resolution in the high M, range of PfEMP1. In some cases (Fig 6), the intact iodinated PRBC were treated for 5 minutes at 20°C with 1 μg/mL TPCK-trypsin (Sigma Chemicals Co, St Louis, MO) in PBS, followed by addition of 1 mg/mL trypsin inhibitor (Sigma Chemicals Co) and 1 minute of further incubation, then washing with PBS before detergent extraction.

**ImmunoeM.** Intact unfixed PRBC were incubated in fluid phase successively with 1/5 dilution of human serum (37°C, 30 minutes), then 1/40 dilution of rabbit anti-HuFcy (Cappel Worthington), then 1/20 dilution of Protein A-Gold (10 nm; Janssen Life Sciences, Piscataway, NJ), with extensive washes at 4°C with PBS between the different antibody incubations. The cells were then fixed for 60 minutes at 23°C with 2% glutaraldehyde, 50 mmol/L sodium phosphate, pH 7.4, 4% sucrose, 0.01 mmol/L CaCl₂, and 0.01 mmol/L MgCl₂. The cells were postfixed with 1% OsO₄ after several washes with 20 mmol/L phosphate buffer, pH 7.4, containing 15 mmol/L NaCl, and dehydrated in a graded series of ethanol solutions. Samples were embedded in Epon 812 and sectioned with a diamond knife on a Porter Blum Mt-2 ultramicrotome. After uranyl acetate and lead citrate staining, sections were...
SURFACE ANTIGEN OF P FALCIPARUM

RESULTS

West African sera show similar surface IFA reactivity with isolates from different geographic regions. A panel of 17 sera from different geographic regions (The Gambia, Ghana, and Nigeria) was tested for surface IFA reactivity with Ghanaian isolate 12, and with Gambian isolate 373 (Table 1). Sera that contained antibodies reactive with infected cells of one isolate also contained antibodies reactive with the other isolate. The two sera (TK669 and AK3) that failed to react with one isolate also failed to react with the other isolate. The immune status of these two individuals was not investigated but it is possible that they were nonimmune semirural dwellers. With one exception (Gambia 758), the immunofluorescence of each serum with one isolate was identical to that with the other isolate, or different by only one doubling dilution, even though the titers of the antisera ranged over five doubling dilutions. Gambian serum 758 reacted much more strongly with the Gambian isolate than with the Ghanaian isolate. Ghanaian serum BL2, which had a high titer with both the Ghana 12 and the Gambia 373 isolate (Table 1), had only a low titer with Gambia 434 (Fig 2).

Characterization of the agglutination reactivity of Gambian isolates with acute, convalescent, and hyperimmune sera. A panel of sera was selected to perform a comparative study of the different methods for detecting infected cell surface antigens. Figure 1 shows the agglutination titers of acute and convalescent serum from patient 434 and three sera (AK11, BL1, and BL2) collected from “immune” Ghanaian adults living in a rural village. Comparison of the agglutination titers of acute and convalescent serum of patient 434 with the homologous 434 isolate shows that isolate-specific agglutinating antibody activity has been acquired by this individual during the 3-week convalescence period after drug treatment. By contrast, the reactivity of the patient’s serum with the four heterologous isolates in the panel remained unchanged through the convalescence period (Fig 1). Serum from patient 434 did not agglutinate two of the four heterologous isolates. By contrast, sera from “immune” Ghanaian adults reacted with all five Gambian isolates, with serum BL1 being of lower titer than the other two examined.

The same panel of sera was used for quantitative studies of agglutination, immunofluorescence, and immunoEM of isolate 434 (Figs 1, 2, 7, 8, and 9). The same sera, and

examined with a JEOL 100CX electron microscope (JEOL USA Inc, Peabody, MA).

West African sera show similar surface IFA reactivity with isolates from different geographic regions. A panel of 17 sera from different geographic regions (The Gambia, Ghana, and Nigeria) was tested for surface IFA reactivity with Ghanaian isolate 12, and with Gambian isolate 373 (Table 1). Sera that contained antibodies reactive with infected cells of one isolate also contained antibodies reactive with the other isolate. The two sera (TK669 and AK3) that failed to react with one isolate also failed to react with the other isolate. The immune status of these two individuals was not investigated but it is possible that they were nonimmune semirural dwellers. With one exception (Gambia 758), the immunofluorescence of each serum with one isolate was identical to that with the other isolate, or different by only one doubling dilution, even though the titers of the antisera ranged over five doubling dilutions. Gambian serum 758 reacted much more strongly with the Gambian isolate than with the Ghanaian isolate. Ghanaian serum BL2, which had a high titer with both the Ghana 12 and the Gambia 373 isolate (Table 1), had only a low titer with Gambia 434 (Fig 2).

Characterization of the agglutination reactivity of Gambian isolates with acute, convalescent, and hyperimmune sera. A panel of sera was selected to perform a comparative study of the different methods for detecting infected cell surface antigens. Figure 1 shows the agglutination titers of acute and convalescent serum from patient 434 and three sera (AK11, BL1, and BL2) collected from “immune” Ghanaian adults living in a rural village. Comparison of the agglutination titers of acute and convalescent serum of patient 434 with the homologous 434 isolate shows that isolate-specific agglutinating antibody activity has been acquired by this individual during the 3-week convalescence period after drug treatment. By contrast, the reactivity of the patient’s serum with the four heterologous isolates in the panel remained unchanged through the convalescence period (Fig 1). Serum from patient 434 did not agglutinate two of the four heterologous isolates. By contrast, sera from “immune” Ghanaian adults reacted with all five Gambian isolates, with serum BL1 being of lower titer than the other two examined.

The same panel of sera was used for quantitative studies of agglutination, immunofluorescence, and immunoEM of isolate 434 (Figs 1, 2, 7, 8, and 9). The same sera, and
others, were used for the immunoprecipitation studies in Figs 3, 4, and 6, but a different parasite isolate was used for these experiments because insufficient blood from any one patient was available.

Quantitative comparison of surface IFA and agglutination reactivity of West African sera with Gambian isolate 434. The titration curves for surface IFA and agglutination of isolate 434 with the panel of human sera are shown in Fig 2. Control serum and the acute homologous serum did not react in either assay at 1/5 serum dilution. The titration curves of each serum with the two different assays are broadly similar, but the curves of different sera are quite different. For instance BL2 has a very high reactivity at 1/5 serum dilution, but titers out rapidly, whereas AK11 is moderately reactive but titers out slowly. Note that in Table 1, BL2 was found to have a high titer with two other isolates.

Identification of knob-associated surface antigens of infected erythrocytes of isolate 434 by immunoEM. In immunoEM (Fig 7) with human serum, rabbit anti-HuFcy, and Protein A-Gold, no labeling of PRBC of isolate 434 was seen with the acute homologous serum, but with convalescent homologous serum and with adult Ghanaian serum labeling of the knobs was seen in a characteristic pattern involving bunches of gold particles above the membrane. This pattern was similar for the two sera. Some bunches of gold particles did not correspond to a visible knob; without taking serial sections it is not possible to conclude whether such bunches correlated with a knob out of the plane of the section.

Quantitative comparison of surface IFA reactivity and immunoEM reactivity of isolate 434 with a panel of West African sera. To detect any quantitative relation between the IFA and immunoEM results, the percentage of cells reactive by IFA, the percentage of knobs reactive in immunoEM, and the percentage of infected cells carrying reactive knobs in immunoEM were determined for several sera (Table 2). These quantitative measures again confirmed the acquisition of surface-reactive antibody during convalescence of patient 434, as well as the reactivity of most Gambian and Ghanaian adult sera with this isolate.
Gambian serum A9 had a lower reactivity than the others. The immune status of this individual was not investigated.

A strong positive correlation ($r = .925, P < .001$) was found between the percentage of reactive knobs in immunoEM and the percentage of infected cells positive in surface IFA (Fig 8). The data suggest that an average of 2% to 3% of knobs must be positive in immunoEM before surface IFA reactivity can be detected in the cell population.

**Blockade of cytoadherence of isolate 434 by human sera.** In a cytoadherence assay in which both the parasitized erythrocytes of isolate 434 and the C32 melanoma cells were preincubated with different sera, neither control serum nor the acute homologous serum of patient 434 were able to inhibit cytoadherence of the infected cells (Fig 9). Preincubation with convalescent serum 434, or with any one of three different adult Ghanaian sera, gave significant inhibition of binding ($P < .05$ in two-tail t-test) (Fig 9). Note that this panel of sera is the same as those in Fig 1, and that the sera that inhibit cytoadherence are also those that agglutinate. In the experiment in Fig 9, approximately 200 PRBC were adherent per 100 C32 cells (Fig 9). Although this level of adherence to C32 cells in vitro is low compared with some other parasites, this was a reproducible measurement for isolate 434, and this level is within the range recorded for wild isolates in the literature.

**Trypsin-sensitive $^{125}$I-surface-labeled antigens.** Purified mature infected erythrocytes from parasite isolates 176, 179, and 220 were surface iodinated and sequentially extracted with TX100 and SDS. SDS-polyacrylamide gel electrophoresis (PAGE) analysis showed several high $M$, iodinated bands in the SDS extract of isolates 176 and 179, whereas isolate 220 contained a single such band (Figs 3, 4, and 6). These bands could only be detected in the SDS extract of infected cells and immunoprecipitates thereof, and not in the TX100 extract and its immunoprecipitates (Fig 3) or in extracts of uninfected cells from the infected culture (Fig 4). Under the conditions used in these experiments, PfEMP2/MESA was not iodinated, because Western blotting with anti-PfEMP2 antibodies identified an antigen of
Fig 6. Trypsin-sensitivity of 125I-labeled cell surface antigens from Gambian isolate 179.

Intact iodinated PRBC of isolate 179 were also treated with low amounts of trypsin before detergent extraction. SDS extracts from trypsin-treated cells no longer contained the same 125I-labeled high M, bands as the untreated cells (Fig 6), showing that the antigen is trypsin-sensitive on the surface of the intact cell. Therefore, by analogy with the results obtained in Aotus-adapted parasites and cultured lines, the iodinated bands will be referred to below as PfEMP1.

Immunoprecipitation of 125I-labeled infected RBC surface antigens by sera of defined agglutination reactivity. Figure 3 shows the immunoprecipitation patterns of 125I-surface-labeled isolate 220 with Ghanaian adult sera, heterologous acute and convalescent serum 434, and a serum pool of European controls. The Ghanaian adult sera were the same as those used in the experiments shown in Figs 1, 2, 7, 8, and 9, but the parasite isolate used was different. All sera tested for immunoprecipitation were also tested for agglutination with this particular isolate. Therefore, although the results of this and following experiments cannot be compared quantitatively with those in Figs 1, 2, 7, 8, and 9, the agglutination test provides an internal control for the surface-reactivity of the sera with isolate 220.

In Fig 3, the ability of the sera to immunoprecipitate PfEMP1 is found in the adult Ghanaian sera but not in the heterologous acute or convalescent serum 434. Furthermore, all six adult Ghanaian sera tested are able to both immunoprecipitate PfEMP1 and agglutinate-infected erythrocytes of isolate 220.

The key finding in Fig 4 is the fact that PfEMP1 of isolate 176 is not precipitated by homologous 176 acute serum, but only by the 176 convalescent serum. This finding correlates with the acquisition of isolate-specific agglutinating activity during the convalescent period (Fig 4). In addition, Fig 4 confirms that adult sera, in this case from The Gambia, immunoprecipitate PfEMP1 from another isolate, 176, and shows that PfEMP1 is immunoprecipitated by a heterologous child serum (197 convalescent) that also agglutinates this isolate.

Figure 5A and B shows the results of screening Ghanaian sera with an Aotus-adapted line of P falciparum, FVO. Of the 17 sera tested, all those that were positive for agglutination (12 of 17) were also positive for immunoprecipitation of PfEMP1, which, in this line of P falciparum, has an M, just below that of the spectrin α-chain. However, of the five sera that were negative for agglutination, only two were also weak or negative in immunoprecipitation, whereas the other three were strongly positive. We did not find any sera that were positive for agglutination but negative for immunoprecipitation.

Table 1. Cross-Reactivity of West African Sera With Isolates From Ghana and The Gambia

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DISCUSSION

In this study we have attempted to characterize the surface antigens of P. falciparum-infected erythrocytes by comparing antigens detected by sera from distinct geographic regions and from acute and convalescent patients, using a range of different assay methods.

Marsh and Howard previously showed that isolate-specific antibodies to the P. falciparum-induced agglutinogen are acquired by Gambian children during convalescence after an acute attack of P. falciparum malaria, while hyperimmune adults have antibodies capable of reacting with many Gambian isolates. We extend these observations here by showing that the wide reactivity of adult sera extends to distinct geographical regions because sera from Nigeria, Ghana, and The Gambia are similarly reactive with two isolates from The Gambia and Ghana (Table 1). This finding may be because of the presence of shared epitopes on these two isolates or because of the large number of different antibody specificities in the adult sera. We further show that the acquisition of isolate-specific surface-reactive antibodies upon convalescence from an acute attack in a nonimmune individual is demonstrated not only by agglutination (Fig 1), but also by surface IFA (Table 2, Fig 2), immunoEM (Fig 7), cytoadherence inhibition (Fig 9), and immunoprecipitation of 125I-labeled surface antigens (Fig 4). The comparison of acute and convalescent sera from the

Fig 7. ImmunoEM of isolate 434 with West African sera. Intact PRBC containing mature trophozoite and schizont-stage parasites were incubated sequentially with antibody and Protein A gold (10-nm particles) then processed for immunoEM. (A) Acute serum from patient 434; (B) 3-week convalescent serum from patient 434; (C) serum BL-2 from Ghana. In (A) and (C) the bar represents 0.1 μm. In (B) the bar represents 0.05 μm.
same individual is therefore a useful tool for the detection of the isolate-specific surface antigen as distinct from other immune reactivity against malaria.

A quantitative correlation was shown between surface IFA titers and agglutination titers of sera with isolate 434. At the highest serum concentration used in Fig 2, sera with higher IFA reactivity also, in general, had higher agglutination reactivity. As has been reported previously, we found that agglutination could be detected at higher dilutions of serum than IFA. However, the IFA and agglutination reactivity of the different sera changed relative to each other at high dilution. This result presumably reflects the polyclonal nature of the sera and the somewhat different properties required of antibodies for the two assays. However, the general similarity in the assays suggests that surface IFA and agglutination detect either a single antigen or a number of coexpressed antigens.

Two parameters were measured in immunoEM: percentage of knobs reactive and percentage of PRBC sections with reactive knobs. The relationship between these two parameters may be in part described by the Poisson distribution and in part determined by nonrandom factors such as heterogeneity in the antibody and knob and cell populations. There is good correlation between the percentage of knobs reactive in immunoEM and the percentage of PRBC reactive in IFA (Table 2 and Fig 8), with a threshold suggesting that a minimum number of knobs may need to bind antibody for a cell to score positive by IFA.

In immunoEM (Fig 7), the majority of label was found associated with knobs, indicating that the surface antigens reactive with the antisera are preferentially localized at the knobs, and that the same pattern of labeling was obtained with the homologous convalescent as with an adult serum. Antigens localized to the knobs have also been detected using the MoAb 4A3, con A-ferritin, and IgG against senescent band 3. The relationship between these antigens and PfEMP1 remains to be established, but it is possible that several surface antigens are localized at the knobs.

Previous studies have suggested a role for a knob-associated surface antigen in cytoadherence, because the cytoadherent phenotype was for a long time only observed in knob-positive parasites. However, two lines of parasites have recently been reported that do not have knobs but are cytoadherent in vitro. Furthermore, Hommel and Semmoff reported that Saimiri immune serum reacted both at knobs and at knob-free areas of the erythrocyte membrane. The present study demonstrates that human sera positive

### Table 2. Reactivity of Human Sera With the Surface of *P. falciparum*-Infected Erythrocytes From Gambian Patient 434

<table>
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<tr>
<th>Serum</th>
<th>Immuno-fluorescence (% of PRBC reactive)</th>
<th>ImmunoEM* (% of knobs reactive)</th>
<th>ImmunoEM* (% of PRBC sections with reactive knobs)</th>
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*There were ~50 knobs per electronmicroscopy section of a PRBC.
for agglutination, surface IFA, immunoEM, and blockade of cytoadherence only react at the knobs of a knob-positive wild isolate.

In this study we obtained blockade of cytoadherence by the homologous convalescent serum and by adult sera, but not by the homologous acute serum (Fig 9). Blockade of cytoadherence by human sera and particularly its relationship to agglutinating activity have been examined in several recent studies. Singh et al compared the ability of two homologous convalescent and acute sera to inhibit cytoadherence of the corresponding Thai isolates; there was no seroconversion during convalescence as measured by cytoadherence inhibition. It is possible that these two convalescent sera did not have agglutinating activity, because this was not assayed. Alternatively, the lack of inhibitory activity may reflect properties of the Thai specimens or the different assay procedure used. Forsyth et al reported only on the activity of convalescent sera and thus did not comment on the acute to convalescent conversion. However, they found both agglutination and cytoadherence inhibition with all homologous convalescent sera tested and obtained a strong correlation between the agglutination and cytoadherence inhibition scores of heterologous sera. Southwell et al also noted a general correlation between agglutination and cytoadherence inhibition activity, although some sera showed cytoadherence inhibition without agglutination activity. Blockade of cytoadherence by agglutinating sera may be caused by agglutination per se, and/or may reflect blocking of the receptor binding site by antibody.

Surface iodination of wild isolates gave between one and three bands of high M, in the SDS extract (PfEMP1), which in one case were shown to be trypsin-sensitive (Figs 3, 4, and 6). The existence of several high M, iodinatable proteins in a single isolate was noted previously, and may reflect a nonclonal population of parasites, the parasite's ability to vary this antigen rapidly, or the expression of multiple PfEMP1-like proteins by a single clone.

In a previous study it was found rare that a child's serum agglutinated a heterologous isolate as well as the homologous one. However, Fig 4 shows that in one such case, with serum 197CO, the same PfEMP1 bands are immunoprecipitated by this serum as by the homologous serum. Furthermore, the same high M, 125I-labeled surface antigens are precipitated by pooled hyperimmune sera as by the convalescent children's sera (Fig 4).

Although all sera tested that were positive for agglutination also immunoprecipitated the 125I-labeled surface antigen, a quantitative relation between immunoprecipitation of the high molecular weight 125I-labeled antigen and the surface property of agglutination was not established. In Figs 3 and 4, the sera used had not, because of the limitations of working with field specimens, been assayed quantitatively for agglutination. A more extensive study with an Aotus-adapted laboratory line (FVO) showed that three of 17 adult West African sera tested showed strong immunoprecipitation of PfEMP1 despite weak or negative agglutination reactivity (Fig 5). There are several possible reasons for the differential reactivity of these sera in the two assays. One group of reasons involves the different epitope and isotype requirements of the two assays: immunoprecipitation detects antibodies reacting with the detergent-solubilized form of the antigen and having isotypes binding to Protein A-Sepharose, whereas agglutination detects antibodies reactive with native antigen exposed on the cell surface and would detect only antibodies of epitope specificities and isotypes that permit the formation of intercellular bridges. The second group of possible reasons for the differential reactivity of the sera in agglutination and immunoprecipitation would invoke the involvement of other antigens in these assays. For instance, agglutination might involve an antigen different from PfEMP1, but antibodies to the agglutinogen would then usually be acquired at the same time as those to PfEMP1. Alternatively, PfEMP1 may be the agglutinogen, but may be immunoprecipitated efficiently by antibodies against a coprecipitating antigen. In this respect it is interesting to note that PfEMP2/MESA, another TX100 insoluble, SDS-extractable antigen, was found to coprecipitate with band 4.1. This array of possible explanations should be examined in future studies.

From the present study, having examined the surface reactivity of a number of sera and isolates by agglutination, surface IFA, immunoEM, blockade of cytoadherence, and 125I-PfEMP1 immunoprecipitation, we conclude that, in general, sera that are surface-reactive by any one technique are also surface-reactive by another technique. In the case of agglutination, surface IFA, and immunoEM, a quantitative correlation could be established. A practical consequence is that use of a single technique (eg, agglutination) to screen sera is likely to be sufficient to detect most surface-reactive sera. Conceptually, the data may imply the existence of a single antigenically important surface antigen, or a set of coexpressed antigens. The data leave room for the existence of quite distinct antigens that may yet be discovered. However, it is clear that infected erythrocyte surface antigens similar by several criteria to PfEMP1 as previously characterized in Aotus-adapted and culture-adapted parasites also occur on the surface of infected human erythrocytes taken directly from West African patients.

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