ANTIGENICITY OF THE INFECTED-ERYTHROCYTE AND MEROZOITE SURFACES IN FALCIPARUM MALARIA*

By SUSAN G. LANGRETH AND ROBERT T. REESE

From The Rockefeller University, New York 10021

Recent advances in techniques for the in vitro cultivation of Plasmodium falciparum have now made it possible to obtain all stages of the asexual erythrocytic life cycle of the parasite (1). The protective properties of antisera from individuals immune to malaria and the antigenicity of parasite components can now be examined in vitro. During the erythrocytic cycle of Plasmodium falciparum two important foreign surfaces are exposed to the host immune system: the infected erythrocyte membrane and the plasma membrane of the parasite at the merozoite stage. This paper reports the results of our experiments on the immunocytochemical localization of antibodies from immune sera on these two surfaces.

Infection with Plasmodium falciparum results in alterations of the host erythrocyte membrane, which develop as the parasite within matures. Most prominent of these are the knobs, electron-dense inverted cuplike protrusions from the erythrocyte surface. These knobs or excrescences arise in both in vivo and in vitro infections (2–6). They are believed to provide receptors by which erythrocytes, containing late-stage parasites, sequester in the capillaries (7, 8). Although the knobs induced by P. falciparum may be antigenically distinct from the rest of the infected erythrocyte surface (8), their reactivity with the sera from animals which are immune to malaria has not been examined. P. knowlesi does not induce knobs but does induce antigenic variant-specific alterations in its host erythrocyte membrane. These alterations have been demonstrated by the agglutination of schizont-infected erythrocytes when treated with sera from monkeys immune to this malaria parasite (9).

Several important questions have not yet been answered about the antigenicity of the P. falciparum-infected erythrocyte surface. Are the parasite-induced alterations which stimulate antibody in the immune host located on the knobs (7, 8) or are other regions of the membrane also involved, such as with P. knowlesi (9)? Do different strains of P. falciparum from different geographic areas induce immunologically cross-reactive alterations of the erythrocyte membrane, or are the alterations strain and/or variant specific? Are the knobs induced by a single strain of parasite antigenically similar in both human and monkey erythrocytes, or is their antigenicity influenced by the host cell?

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The surface of the merozoite, the invasive form in the asexual erythrocytic cycle of *P. falciparum*, is believed to be important immunologically because merozoite-rich fractions of parasite material have been shown to induce protective immunity in rhesus and owl monkeys (10-14). In addition, Miller et al. (15) demonstrated that rhesus immune serum agglutinated *P. knowlesi* merozoites by cross-linking their cell coats and also reduced the ability of merozoites to invade new erythrocytes.

We have examined merozoites and the surfaces of both human and *Aotus* erythrocytes infected with *P. falciparum* by immunoelectron microscopy. We found that different strains of *P. falciparum* induce cross-reactive modifications of their host erythrocyte surface (knobs) which are antigenically similar regardless of whether the erythrocyte is of monkey or human origin. Antibody bound to the knobs did not fix enough complement to lyse the infected erythrocyte but may play a role in opsonization. In addition, common merozoite surface antigens were observed in heterologous African strains of *P. falciparum*.

### Materials and Methods

**Parasites.** Three strains of the human malaria parasite *Plasmodium falciparum* were used (16): FCR-1/FVO, chloroquine-resistant, from Vietnam; FCR-3/FMG, chloroquine sensitive, from the Gambia, West Africa; and FCR-4/6252, chloroquine sensitive, also from the Gambia. Both knob-producing (K+) and knobless (K-) variants of strain FCR-4 were examined (17). Parasites were grown in human erythrocytes by established methods (1, 18). Subcultures were started at 0.1-0.2% parasitemia in 4-6% erythrocyte suspensions and collected when the parasitemias were 6-8%, usually on the 4th d. Uninfected human erythrocytes were also cultured for 4 d to serve as controls. Naturally released merozoites were obtained by harvesting the supernatant culture medium from a 2.5-h incubation of schizont-infected cells (19). During this incubation, the erythrocytes were resuspended every 30 min to minimize reinvasion. *Aotus* monkeys were infected with *Plasmodium falciparum* strain FCR-3 as described previously (14). Blood was drawn from infected monkeys when the parasitemias were 10-50%.

**Animals and Sera.** *Aotus* monkeys of karyotypes I, II, III, and IV (14) were made immune to malaria either by infection followed by drug treatment or by immunization with parasite material obtained from in vitro cultivated cells (14). Two monkeys (M-9 and M-73) were able to control even their initial contact with parasites so that drug treatment was unnecessary. The details on the treatment of each animal are found in Table I. Immunity to malaria was demonstrated in vivo and in vitro. Monkeys were considered immune when the maximum parasitemia resulting from intravenous challenge with large numbers (10⁷) of parasitized monkey erythrocytes was <0.4%. (Naive *Aotus* of karyotypes II, III, and IV are generally killed within 3 wk after such a challenge.) Unless otherwise stated, immune sera were drawn beginning ~1 mo after the second parasite challenge. Sera from animals 72, 73, 75, and 81 were pooled; sera from animals M-9, M-73, M-90, and M-94 were used separately. Sera from immune animals inhibited in vitro growth of the parasite (18, 20). Normal human serum was obtained from the New York Blood Center. All sera used in this study were heat inactivated at 56°C for 30 min, absorbed with human erythrocytes, and frozen till used. Goat anti-human immunoglobulin (Ig) conjugated to ferritin was purchased from N. L. Cappel Laboratories Inc., Cochranville, Pa.

**Immune Labeling.** Infected erythrocytes, uninfected erythrocytes, and merozoites were washed three times in buffered RPMI-1640 (1) at 18°C before exposure to antisera. Aliquots containing 12 μl of packed cells were mixed with 50 μl of *Aotus* serum. The sera were used either undiluted or at 1:2 or 1:4 dilutions with RPMI buffer. Incubations were for 10 min on ice with gentle agitation. The cells were then washed three times with ice-cold buffered RPMI-1640. The second antibody, goat anti-human Ig conjugated to ferritin, was then added (50 μl diluted 1:1 with buffer). After thorough mixing, incubation was again for 10 min on ice. The samples were then washed three times in ice-cold buffered RPMI-1640 and fixed for electron microscopy.

Controls included incubation with normal serum (human and monkey), incubation with
Table I
Treatment of Monkeys Used as a Source of Immune Serum

|   | Karyotype | Infection Details                                                                 |
|---|-----------|-----------------------------------------------------------------------------------|
| M-9 | I; female | infected with FCR-1, parasitemia increased to 10% before recovery, spleenectomized 5 mo later and after month challenged with 2 × 10⁹ FCR-1 injected intraperitoneally, maximum parasitemia 0.1%. Challenged 47, 50, and 54 mo later with 1.2 × 10⁸, 5 × 10⁸, and 2 × 10⁹ FCR-1; maximum parasitemias 1%; <1% and <0.01%, respectively. Challenged 9 mo after last FCR-1 challenge with 7 × 10⁹ FCR-3; maximum parasitemia <0.01%. Sera used for this study were obtained 1 mo after the last challenge with FCR-1 and 2 mo after challenge with FCR-3. |
| M-72 | IV; female | infected with 1.9 × 10⁸ FCR-1 strain; day 10 parasitemia 13%; treated with 10 mg mefloquine; cured. Challenged with 5 × 10⁷ FCR-3 strain 9 mo after initial challenge; maximum parasitemia <0.2%. Challenged with FCR-3 strain again 12 mo after initial challenge. |
| M-73 | II; female | infected with 2.5 × 10⁸ FCR-1 strain; maximum parasitemia 2%, cured by itself without drugs; 10, 17, and 20 mo later challenged with 5 × 10⁷ FCR-3 strain; parasitemias <0.1%. |
| M-75 | III; female | infected with 1.9 × 10⁸ FCR-1 strain; day 10 parasitemia 13%; treated with 10 mg mefloquine; cured. Challenged with 2.5-5 × 10⁵ FCR-3 strain 4, 8, and 12 mo after initial challenge; parasitemias did not exceed 0.4%. |
| M-81 | II; female | immunized as described (14), challenged with 5 × 10⁷ FCR-3; parasitemia <0.4%; challenged 5 mo later with 10⁵ FCR-3 strain; parasitemia <0.1%. |
| M-90 | III; male | infected with 1.9 × 10⁸ FCR-1 strain; day 9 parasitemia 16%; treated with 10 mg mefloquine; cured. Challenged with 5 × 10⁷ FCR-3 strain 8 and 12 mo after initial challenge. |
| M-94 | II; female | infected with 2.5-5 × 10⁷ FCR-3 strain 4, 8, and 12 mo after initial challenge. |

All infections and challenges were with parasitized monkey erythrocytes. All strains were K+. (17).

only the second ferritin-conjugated antibody, and blocking of the second antibody by its prior incubation with Aotus immune serum before exposure to cells which had been incubated with the first antibody.

Electron Microscopy. The samples were resuspended in 0.5 ml buffered RPMI-1640 and 7 ml of glutaraldehyde fixative (2% glutaraldehyde, 0.1 M Na cacodylate-HCl buffer, pH 7.4, 0.12 M sucrose, 0.2 μM CaCl₂) was added. Further processing for electron microscopy was as described previously (6). Stained thin sections were examined in a Philips 300 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N. J.) operated at 60 kV.

Lysis of Parasitized Erythrocytes with Antiserum and Complement. Attempts were made to lyse parasitized erythrocytes using immune monkey serum and either normal human or normal monkey serum as a source of complement. Parasites which produce knobs (K+) and variants of these same strains which do not induce knobs (K-) were studied (17). Parasitized cells were washed with buffered RPMI-1640 (1) and then incubated with complement and immune monkey serum containing anti-K antibodies. Sera used as a source of complement were absorbed with human A+ cells in an ice bath and stored in aliquots at −72°C.

To increase the probability of lysing parasitized cells, some of the infected erythrocytes, which had first been incubated with anti-K antisera, were washed and then incubated with rabbit anti-human Ig. Guinea pig serum was added as a source of complement. To control for lysis not mediated by complement, K+ parasitized erythrocytes were incubated with heat-inactivated (56°C, 30 min) sera. In addition, anti-K antisera were incubated with erythrocytes infected with K− parasites. Because K− parasites do not induce knobs in the erythrocyte membrane and, as will be shown, bind no antibodies from immune sera on their erythrocyte surfaces, they provide an excellent control to assess lysis as a result of manipulation of the cells.

For positive controls, normal and infected erythrocytes were incubated with either monkey antibody to human A+ erythrocytes and monkey complement or rabbit antibody to human A+ cells and guinea pig complement.
## Table II

**Antibody Binding to Parasitized Erythrocyte Surface**

| Specificity of Aotus serum | Parasite strain and host erythrocyte | Uninfected human | FCR-1 human | FCR-3 human | FCR-4 human | Uninfected Aotus | FCR-3 Aotus |
|---------------------------|--------------------------------------|------------------|-------------|-------------|-------------|-----------------|-------------|
| Anti-FCR-1                |                                      | K                | K           |             |             | -               | -           |
| Anti-FCR-3                |                                      | K                | K           | K           |             | -               | -           |
| Anti-FCR-1 + FCR-3        |                                      | K                | K           | K           | K           | -               | -           |
| Normal                    |                                      | -                | -           | -           | -           | -               | -           |

K = knobs on the erythrocyte surface. (--) = no binding detected. Blank means experiment not performed.

Human erythrocytes were cultured in vitro; Aotus erythrocytes came from in vivo infections.

## Results

**Infected Erythrocyte Surface.** Table II summarizes the results of our experiments to locate antibodies from immune sera which are bound to the parasitized erythrocyte surface. Antibody is localized exclusively on the knobs. The binding of antibody to the knobs of strain FCR-1 schizonts, as detected by the ferritin label, is similar with three different types of antisera: antiserum from animals exposed to two different strains of parasite (anti-FCR-1 and FCR-3, Fig. 1), antiserum from animals exposed to a different strain of the parasite (FCR-3, Fig. 2), and antiserum from animals exposed to the same parasite strain (FCR-1, Fig. 3). There is no binding to the knobs of an FCR-1 schizont which has been incubated with normal Aotus serum (Fig. 4).

Bound antibody is detected on the knobs of parasitized erythrocytes of both monkey and human origin (Figs. 5–7). Thus, the parasite can induce alterations which are antigenically similar even though its host cell differs. The antibodies interact with the knobs regardless of whether the cells examined have come directly from an animal or from in vitro cultures. This demonstrates that the antisera are not simply reacting with membrane structures which are normally not manifested and are exposed (or created) by in vitro cultivation. It also shows that the knobs are recognized by the immune system of the monkey as the parasite matures in its host erythrocyte. Extensions of knob material which later may be sloughed have been observed on parasitized erythrocytes both from animals and from culture (6). These structures bind antibody (Figs. 1 insert, 7). Once sloughed, this knob material may undergo

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**Fig. 1.** Human erythrocyte infected with *P. falciparum* strain FCR-1. The antiserum is from a monkey (M-9) immune to strains FCR-1 and FCR-3. Bound antibody is detected by reaction with a second antiserum, goat anti-human Ig conjugated to ferritin. The ferritin is found associated with the knobs. The inset shows a monkey erythrocyte, infected with strain FCR-3. The monkey (M-9) antiserum is anti-FCR-1 and anti-FCR-3. Sloughing knob material also binds antibody. Bar = 0.5 μm. × 47,000.

**Fig. 2.** Human erythrocyte infected with strain FCR-1 (Vietnamese). The monkey (M-94) antiserum is anti-FCR-3 (West African). Antibody is detected on the knobs. × 47,000.

**Fig. 3.** Human erythrocyte infected with strain FCR-1 reacted with Aotus antiserum produced by immunization with the same strain (Aotus M-9 before challenge with strain FCR-3). Even with homologous antiserum the amount of ferritin binding per knob is variable. × 47,000.

**Fig. 4.** Human erythrocyte infected with strain FCR-1. The serum is from a monkey which had not been exposed to malaria. A ferritin grain is rarely found. × 47,000.

**Fig. 5.** Human erythrocyte infected with strain FCR-3. The monkey (M-9) antiserum is anti-FCR-1 and anti-FCR-3. The knobs bind antibody. × 47,000.
minor antigenic changes. However, although sloughed material may play a major role in stimulating antibody production, our results show that at least some of the antigens are expressed on the intact membrane of the parasitized erythrocyte in the animal.

The specificity of the interaction of antibody with the knobs on late-stage parasitized erythrocytes has been demonstrated in several ways. Erythrocytes infected with young parasites (ring stage) do not have knobs on their surfaces. Incubation of these cells with serum from immune animals followed by incubation with goat anti-human Ig conjugated to ferritin results in no detectable ferritin label on the erythrocyte surface (Fig. 8). This finding indicates that no antimalarial antibody was bound. Uninfected erythrocytes treated similarly also bind no antimalarial antibody (Fig. 9). The knobs of infected erythrocytes (human and monkey) incubated first with normal sera from monkeys which had never been exposed to malaria and then with anti-human Ig conjugated to ferritin had no ferritin label (Fig. 4). No antibody was detected on infected erythrocytes (K +) when the serum tested was from a splenectomized monkey that died of malaria on the day the serum was drawn. Incubation with only the goat anti-human Ig conjugated to ferritin resulted in no labeling of the infected erythrocyte.
Figs. 10 and 11. Human erythrocytes infected with strain FCR-4. The monkey (M-90) antiserum is anti-FCR-3. Although both parasites are schizonts, the one in Fig. 11 is a knobless variant. Ferritin grains are found on 3 of the 4 knobs in Fig. 10, but no ferritin is found on the knobless erythrocyte in Fig. 11. Bar = 0.5 μm. × 47,000.

Antibody from monkeys immune to malaria does not bind to the surface of parasite variants (K−) which no longer produce knobs. K− variants have now been isolated from several strains of *P. falciparum* which have been maintained in vitro for 2–3 yr (17). Concomitant with the loss of knobs in these variants is the loss of antibody binding to the infected erythrocyte surface. Binding of anti-FCR-3 serum to K+ and K− schizonts of strain FCR-4 is compared in Figs. 10 and 11.

The immunocytochemical sandwich technique employed here does not permit us to quantify the amount of antibody from the various immune sera which bound to the knobs, although we did observe that the amount of ferritin-antibody label varied on adjacent knobs. Some knobs had no ferritin grains on an otherwise well-labeled erythrocyte (Figs. 1 inset, 3, 6, and 10) and the percentage of labeled knobs per erythrocyte varied from 10 to 100% within a sample, even among parasites at the same stage of development.

**Merozoite Surface.** Table III summarizes the results of our experiments on the immunocytochemical localization of antibodies from immune sera on the merozoite surface. Merozoites of *Plasmodium falciparum* have fine brushy cell coats (6, 21). When incubated with sera from monkeys immune to either homologous or heterologous strains, merozoites were agglutinated by the cross-linking of their cell coats. The coats
Table III
Antibody Binding to Parasite Surface

| Specificity of Aotus serum | Parasite strain and host erythrocyte | FCR-4 human | FCR-3 human | FCR-3 Aotus |
|---------------------------|--------------------------------------|-------------|-------------|-------------|
| Anti-FCR-3                | MC (thickened), RB, FP               | MC (thickened), RB, FP | MC (thickened), RB, FP | MC (thickened), FP material |
| Anti-FCR-1 + FCR-3        | MC (thickened), RB, FP               | MC (thickened), RB, FP | MC (thickened), RB, FP | MC (thickened), FP material |
| Normal                    | Little to no ferritin bound, MC usually bound, MC usually absent | Little to no ferritin bound, MC usually bound, MC usually absent | Little to no ferritin bound, MC usually bound, MC usually absent | Little to no ferritin bound, MC usually bound, MC usually absent |

MC = merozoite cell coat. RB = residual body. FP = erythrocyte-free parasite. Human erythrocytes were cultured in vitro; Aotus erythrocytes came from in vivo infections.

lost their bristle-like structure and appeared thickened (Figs. 12–14). A heavy concentration of ferritin-labeled antibody was distributed throughout this altered coat material. The thickness of the coat-antibody complex was variable and could not be correlated with any orientation of the merozoite (Figs. 12–14). Merozoites incubated with normal serum bound little or no ferritin (Fig. 15). In fact, the cell coat was usually absent in these control preparations. All merozoites in these experiments had swollen slightly and suffered some loss of ultrastructural detail by the completion of the serum incubations and washings. Nevertheless, the differences between merozoites incubated with normal or immune serum were remarkable. The residual body, the hemozoin-containing parasite material not included in merozoites, also has a coat (6), and it also binds antibody from immune sera. In addition, we found ferritin-antibody label on erythrocyte-free parasites and broken parasite material.

Leucocytes. Most human and monkey blood samples used in these experiments contained a few leucocytes. Macrophages with phagosomes containing the malarial pigment, hemozoin, were frequently observed. Some of these macrophages also had ingested erythrocyte-free parasites. In samples that had undergone immunocytochemical incubations, we occasionally found a macrophage with as many as nine knob-bearing infected erythrocytes interdigitating with its pseudopods. Most infrequent was the finding of a leucocyte that had ingested an infected (K+) erythrocyte which had been opsonized during the immunocytochemical incubation (Fig. 16). The knobs of such an erythrocyte had bound antibody and were the points of attachment between the ingested erythrocyte and the phagocytizing cell.

Complement. To test whether immune monkey serum containing antibodies to the knobs can activate enough complement to cause specific lysis of K+ parasitized cells, aliquots of parasitized cells were incubated with immune serum and complement. The percentage of trophozoites, schizonts, and segmenters on Giemsa-stained thin films of these preparations did not appreciably change, regardless of whether K+ or K− variants were incubated with immune monkey serum in the presence of human or monkey complement or with immune monkey serum that had been heat-inactivated. Because specific lysis was not observed directly, a rabbit anti-human Ig reagent which had been shown to cross-react well with monkey Ig was added as an enhancing antibody. In the presence of guinea pig complement, this reagent also caused no specific lysis. Parasitized, as well as normal, erythrocytes were lysed when incubated with either monkey antibody to human A+ cells and monkey complement or rabbit...
Figs. 12–14. Merozoites of strain FCR-4. The monkey antisera are from animals (M-9 and M-73) immune to both strains FCR-1 and FCR-3. The cell coats of the merozoites appear thickened, agglutinated, and heavily bind antibody. Bar = 0.5 μm. × 47,000.

Fig. 15. Merozoite of strain FCR-4 treated with serum from a monkey never exposed to malaria. Little ferritin is present. The cell coat of the merozoite may have been lost during the antibody incubation and washes. × 47,000.
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antibody to human A+ cells and guinea pig complement. Thus, if sufficient amounts of the appropriate antibodies are bound to the erythrocytes, they may be lysed by complement. However, human erythrocytes are not easily lysed by antibody and complement. The monkey anti-human erythrocyte serum which had an agglutination titer of 1:1,600 failed to cause lysis when diluted as little as 1:100 (M. R. Motyl and R. T. Reese. Unpublished material.).

Discussion

The binding of antibodies from the sera of monkeys immune to malaria to the knob protrusions of both human and monkey parasitized erythrocytes suggests that
the malaria parasite has induced similar antigenic changes in the membranes of the erythrocytes of both species. This antigenic change in the erythrocyte surface occurs both in vivo and in vitro. It is correlated with the appearance of knobs. All young parasites (ring stage) and all ages of parasites of knobless variant lines (17) have no visible alterations on their host cell surfaces, and no detectable antibody from immune sera is bound to these surfaces (Figs. 8 and 11). Absence of the plaques of electron-dense material called knobs is correlated with absence of this knob antigen.

The knobs produced in cultured human erythrocytes by the three strains of *P. falciparum* examined here have some common antigenicity as determined by our immunocytochemical binding assay. This finding is particularly significant in comparing Southeast Asian (FCR-1) and African (FCR-3 and FCR-4) strains.

The erythrocyte surface of *P. knowlesi* schizonts is capable of undergoing antigenic variation (9). We do not yet know whether the *P. falciparum*-infected erythrocyte surface undergoes similar antigenic variation, which has been suggested (22), and/or whether there are strain-specific knob antigens. The variability in the amount of ferritin label bound and in the number of labeled knobs per erythrocyte may indicate antigenic differences among the knobs of individual parasitized erythrocytes as well as differences which may occur on the same erythrocyte. The loss of knobs in parasite lines which have been in long-term culture (17) could be regarded as an extreme form of antigenic variation of the infected erythrocyte surface.

In vivo the knobs are believed to be involved in the sequestration of older parasites in the deep vasculature of the heart and other organs, where schizogony and segmentation occur (7). These membrane alterations have been shown to be the points of adherence of infected erythrocytes to endothelial cells (3–5). It is apparent that the knobs are under parasite control because these structures are present regardless of whether the parasite is in a monkey or a human erythrocyte. If the antibodies in immune sera are detecting a receptor generated by the parasite so that it can be sequestered during late-stage development, it is not surprising that all parasitized erythrocytes share these same structures. Possession of these structures would be a prerequisite for the interaction of parasitized cells with venule walls.

Although the knobs have been shown to be antigenic (8, 23, and present study), their function in the immune response to malaria remains unclear. During an immunologic crisis, late-stage infected erythrocytes may be found in the peripheral circulation. These crisis forms are believed to be damaged by some antibody-mediated mechanism (24). It is possible that binding of antibody to parasite-induced alterations in the erythrocyte surface may alter the flow of molecules and ions across the erythrocyte membrane thus potentially restricting the metabolism of the parasite. This hypothesis is being tested by measuring the ability of serum from immune monkeys to inhibit the in vitro growth of K+ and K– strains of the parasite (M. R. Motyl and R. T. Reese. Unpublished material.). A second possibility is that antibodies bound to the parasitized erythrocyte membrane may fix complement, leading to adverse effects on the parasite. However, our results show that very few parasitized cells are ruptured by the direct action of complement, even though complement may be fixed by antibodies bound to the parasitized erythrocyte surface.

As early as 1936, it was suggested that the phagocytosis of *P. brasilianum*-infected cells was assisted by opsonizing antibody (25). Focal junctions formed between the knobs of *P. brasilianum*-infected erythrocytes and both Kupffer cells and macrophages
have been observed (26). In *P. knowlesi* infections, a correlation was found between the decrease in parasitemia in the peripheral circulation and an increase in the opsonizing activity of the host serum (27). We have occasionally observed infected monkey erythrocytes which have been opsonized (antibody-ferritin bound on the knobs) and then phagocytized by monkey leucocytes during our immunocytochemical incubation (Fig. 16). Phagocytosis of erythrocytes occurs in avian, rodent, and primate malarias. However, selective phagocytosis of parasite-infected erythrocytes has not been demonstrated (28). Nevertheless, peripheral blood leucocytes from *Aotus* monkeys inhibit the in vitro growth of *P. falciparum* (29). Further experiments are being conducted to determine the mechanism(s) of leucocyte inhibition and the significance of antibody-knob-leucocyte interactions.

When treated with immune serum, the cell coats of *P. berghei* and *P. cynomolgi* sporozoites appeared thickened and bound antibody (30). Agglutination of merozoites by their cell coats as a result of incubation with immune serum has been observed with *P. knowlesi* erythrocytic merozoites (15). Miller et al. (15) also reported that both agglutination and reduction in invasiveness of merozoites were greater with antisera against homologous strains of *P. knowlesi* than with antisera against heterologous strains. We were unable to detect differences in the amount of immune agglutination of merozoites of homologous (FCR-3) or heterologous (FCR-4) strains of *P. falciparum*, which suggests that these merozoites possess many common antigens. However, it should be noted that both FCR-3 and FCR-4 are West African strains. Nevertheless, reports of the protection of merozoite-vaccinated monkeys against challenge by a heterologous strain (10, 12, 18) also suggest that merozoites share significant common antigens.

**Summary**

The antigenicity of altered structures induced by *Plasmodium falciparum* in the membranes of infected *Aotus* monkey and human erythrocytes was examined. Antisera were obtained from monkeys made immune to malaria. Bound antibodies were shown to be localized on the knob protrusions of infected erythrocytes of both human and monkey origin and from both in vitro and in vivo infections. Therefore, *P. falciparum* infection has produced similar antigenic changes in the erythrocyte surfaces of both man and monkey. Uninfected erythrocytes and all knobless-infected erythrocytes bound no antibody from immune sera. Strains of *P. falciparum* from widely different geographic areas that were cultured in vitro in human erythrocytes induced structures (knobs) which have common antigenicity. Merozoites were agglutinated by cross-linking of their cell coats when incubated with immune sera. The binding of ferritin-labeled antibody was heavy on the coats of both homologous and heterologous strains of the parasite, indicating that the merozoite surfaces of these strains share common antigens.

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