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Antibodies to a histidine-rich protein (PfHRP1) disrupt spontaneously formed *Plasmodium falciparum* erythrocyte rosettes

(malaria)

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ABSTRACT **Cerebral involvement in *Plasmodium falciparum* malaria is associated with sequestration of infected red blood cells and occlusion of cerebral vessels. Adhesion of infected erythrocytes along the vascular endothelium as well as binding of uninfected erythrocytes to cells infected with late-stage asexual parasites (rosetting) may be important in erythrocyte sequestration. We report that the recently discovered rosetting phenomenon shares characteristics with other human cell–cell interactions (heparin sensitivity, temperature independence, Ca^{2+}/Mg^{2+} and pH dependence). Mono- and polyclonal antibodies specific for PfHRP1, a histidine-rich protein present in the membrane of *P. falciparum*-infected erythrocytes, disrupt rosettes but do not affect attachment of infected erythrocytes to endothelial cells. The inhibitory anti-PfHRP1 antibodies reacted with rosetting parasites in indirect immunofluorescence and with *P. falciparum* polypeptides of M_r 28,000 and M_r 90,000 in immunoprecipitation and immunoblotting, respectively. No inhibitory effects on erythrocyte rosetting were obtained with antibodies to related histidine-rich or other antigens of *P. lophurae* or *P. falciparum*. Whether the epitope that mediates rosetting, and is recognized by the anti-PfHRP1 antibodies, is located on PfHRP1 or on a crossreactive antigen remains to be established. The results suggest that endothelial cytoadherence and erythrocyte rosetting involve different molecular mechanisms.**

Erythrocytes infected with mature asexual stages of the human malaria parasite *Plasmodium falciparum* do not appear in the peripheral circulation but are sequestered, particularly in postcapillary venules. In the brain, this sequestration can impede the blood flow and oxygen delivery, leading to cerebral malaria, the most severe form of the disease (1–3). Cytoadherence of infected erythrocytes to the vascular endothelium and binding of uninfected erythrocytes to cells infected with late-stage asexual parasites (rosetting; Fig. 1) have been suggested as the underlying mechanisms (4, 5). Knoblike protrusions on the surface of infected erythrocytes have been considered necessary for endothelial cytoadherence (6–8). Sequestration of immature *P. falciparum* gametocytes has, however, been shown to occur independently of knob protrusions (9). Similarly, sequestration of erythrocytes containing asexual stages in other malarias (*P. knowlesi*, *P. berghei*, *P. chabaudi*) may also occur in the absence of knobs, whereas in yet other malarial species (*P. malariae*, *P. brasilianum*), infected erythrocytes express knobs but do not cytoadhere (10). Recently, *P. falciparum*-infected erythrocytes were found to show cytoadherence in the absence of knobs (5, 11). Moreover, rosetting occurs both in the presence and in the absence of knobs (5).

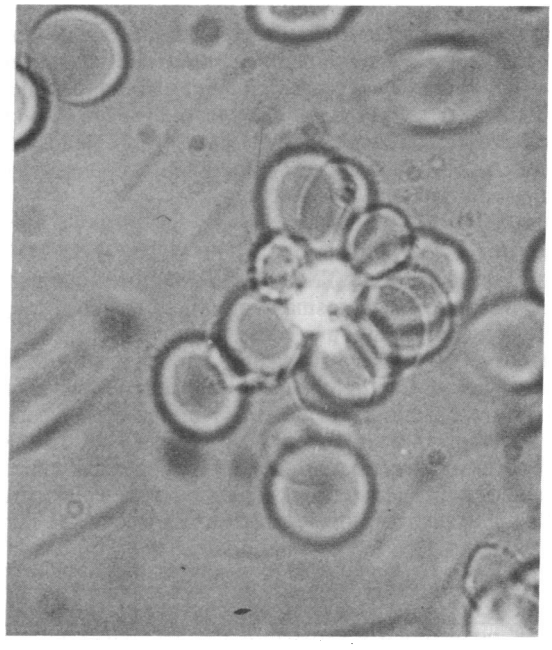

FIG. 1. Rosetting—i.e., spontaneous binding of uninfected erythrocytes to *P. falciparum*-infected erythrocytes. The parasites were visualized in the fluorescence microscope by staining with acridine orange.

Rosetting—i.e., binding of uninfected erythrocytes to infected ones—was first observed in *P. falciparum* malaria (4, 5, 12). More than 10 uninfected erythrocytes are sometimes found adhered to a single infected erythrocyte, but larger agglutinates can also be seen including as many as 20 infected and 40–50 uninfected erythrocytes. Transmission electron microscopy shows that the membranes of the infected and surrounding uninfected erythrocytes are closely adhered. In addition, free parasites are sometimes seen between the cells. Rosetting parasites are found in varying numbers both in established laboratory strains and in fresh isolates from patients. The rosetting phenotype (R^+) seems to be genetically stable, as cloned or enriched parasites kept *in vitro* do not lose the rosetting capacity (5). Rosetting occurs also with *P. chabaudi* and *P. fragile* late-stage infected erythrocytes (4, 13). In the simian malaria *P. fragile*, sequestration of infected erythrocytes is probably due to extensive rosetting. Interestingly, both rosetting and sequestration are modulated by the spleen and are markedly reduced in splenectomized

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Abbreviations: mAb, monoclonal antibody; R^+ (R^-) phenotype, rosetting (nonrosetting) phenotype; K^+ (K^-) phenotype, knobby (knobless) phenotype.

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animals, as is sequestration in *P. falciparum*-infected monkeys (13, 14). Here we describe some of the molecular characteristics of spontaneous rosetting in the human malaria *P. falciparum* and suggest a role for this cell–cell interaction in erythrocyte sequestration and the pathogenesis of cerebral malaria.

MATERIALS AND METHODS

Parasites. *P. falciparum* isolates (Palo Alto, Uganda; R^+PA1, a cloned rosetting parasite obtained from the Palo Alto strain; R^-PA; parasites enriched for nonrosetting cells, obtained from the Palo Alto strain; R^+TM180, enriched for rosetting cells from TM180, Thailand, a strain recently isolated from a patient with acute malaria; R^-F32, Tanzania; R^-FCR3, Gambia) were kept in continuous *in vitro* culture according to standard procedures (15) with heat-inactivated human serum added to the buffered medium. Enrichment on Ficoll-Isopaque and cloning by limiting dilution of the *P. falciparum* strains were as described elsewhere (5). The F32 and FCR3 strains and the parasites from the Palo Alto strain (cloned or enriched) were all of the knobless (K^-) phenotype, whereas the TM180 strain was of the knobby phenotype (K^+) (unpublished data).

Rosetting. Studies were performed on cultures with 5% hematocrit, 4–5% parasitemia at late stage, and a 70–80% (R^+PA1) or 40–55% (R^+TM180) rosetting rate. The parasite culture was mixed with a small amount of acridine orange, a sample was mounted on a glass slide, and rosettes were counted over 50 consecutive vision fields viewed with a ×40 lens in incident UV light (Leitz Laborlux K microscope). Fields were examined diagonally over the slide, from one corner to the other, in order to compensate for an uneven distribution of rosettes on the glass. Infected erythrocytes that had bound two or more noninfected ones were scored as rosettes, and the rosetting rate was expressed as the number of infected erythrocytes in rosettes relative to the total number of late-stage (trophozoite and schizont)-infected erythrocytes.

pH, Ion, and Temperature Dependence of Rosette Formation. The pH of standard culture medium was manipulated by varying the concentration of $NaHCO_3$ and the rosetting rate of the R^+ clone R^+PA1 was assessed in 50-μl aliquots at eight different pH values in the range 5.3–8.3. The chelating agent EDTA or sodium citrate was added to the culture in various concentrations (3.1–100 mM and 4–64 mM, respectively) and assessment was made as mentioned above. In some cases Ca^{2+} (10–80 mM) or Mg^{2+} (10–80 mM) was added to cultures depleted of rosettes by EDTA or sodium citrate. Rosetting parasites were incubated at 4°, 21°, 37°, or 41°C for 60 min to assay for the sensitivity of the rosettes to different ambient temperatures.

Reversal of Rosette Formation. Aliquots (25–50 μl) of the cloned parasite R^+PA1 were mixed with various dilutions of human or mouse monoclonal antibodies (mAbs), rabbit antisera, or purified immunoglobulin preparations and incubated in a 96-well microtiter plate (Linbro), covered with Parafilm and lid, at 37°C for 60 min prior to assessment of rosetting. The rosetting rate of each well was compared to that of a control with normal serum as additive. Studies with anti-PfHRP1 antibodies and heparin were also performed with the R^+TM180 strain. Serum and antibody preparations are described in Table 1.

Indirect Immunofluorescence. Monolayers of erythrocytes infected with rosetting (R^+PA1 and R^+TM180) or nonrosetting (R^-F32 and R^-FCR3) parasites were acetone-fixed and used as antigen. Slides were incubated sequentially for 30 min with dilutions of the anti-PfHRP1, anti-PfHRP2, anti-PfHRP3, or anti-PfEMP2 antibodies (see above; dilutions 1:10 to 1:5120), biotinylated goat antibodies to mouse IgG (Vector Laboratories), and avidin conjugated with fluorescein isothiocyanate (Vector Laboratories).

Table 1. Sera and antibody preparations used in the assays

Antigen*	Antibody†	Ref.
PfHRP1/KP/KAHRP (histidine-rich protein 1/ knob protein/knob-associated histidine-rich protein)	mAb 89 (IgG2a; ascites or purified IgG)	16
	Serum MC1	17
PfHRP2 (histidine-rich protein 2)	mAb 2G12 (IgG1; ascites)	10
	mAb 1D6 (IgG1; ascites)	10
PfHRP3/SHARP (histidine-rich protein 3/small histidine- and alanine-rich protein)	Sera 4132 and 4133, mixed 1:1	10
PlHRP (*P. lophurae* histidine-rich protein)	Serum 6211	17
Pf155/RESA (ring-infected erythrocyte surface antigen, M_r 155,000)	Serum R 22 to synthetic peptide $(EENV)_4$	18
	Serum R 24 to synthetic peptide EENVEHDA	18
Ag332	Human mAb 33G2 (IgM; Con A-purified)	19
	Serum R 45 to synthetic peptide SVTEEIAEEDKSVIEEAV	20
Ag11.1	Serum to synthetic peptide P20, $PEE^{L}_{V}VEEV^{I}_{V}PEE^{L}_{V}VEEV^{I}_{V}GK$	20
PfEMP2/MESA (erythrocyte membrane protein 2/mature erythrocyte surface antigen)	mAb 4H9.1 (IgM; ascites)	10
	mAb 8B7.4 (IgG1; ascites)	10
	Serum 1054	10
GBP (glycophorin-binding protein)	Serum R 071	21
CARP (clustered asparagine-rich protein)	Serum 333 to synthetic peptide CGFNTHEEALNVLKNIKNLIDTSGC	22
FIRA (falciparum interspersed repeat antigen)	Serum R 056	21
S antigen, FC-27	Serum R 748	21
	Serum R 775	21
HSP (heat shock protein, M_r 70,000)	Serum R 284	21
TRAP (thrombospondin-related anonymous protein)	Serum 84 to synthetic peptide GVWDEWSPCSVTCGKGTRSRK	23

Pf, *P. falciparum*.
†mAbs are murine unless otherwise indicated; sera were produced in rabbits.

Immunoblotting. Membrane preparations of Percoll-enriched infected erythrocytes were electrophoresed in SDS/polyacrylamide gels and blotted to nitrocellulose paper. The blots were probed with various antisera or mAbs [anti-PfHRP1 (mAb 89 or rabbit antiserum MC1), anti-PfHRP2 (mAb 1D6), anti-*P. lophurae* HRP (rabbit antiserum 6211)] or control sera and antibodies of the same isotypes, diluted 1:200. Bound antibodies were detected by alkaline phosphatase-conjugated rabbit anti-mouse Ig or sheep anti-rabbit Ig with 1-naphthyl phosphate (Sigma) as substrate and fast blue salt (Merck) as indicator (24).

Immunoprecipitation. Metabolic labeling was done with [^{3}H]histidine (Amersham; 100 μCi/ml of medium; 1 μCi = 37 kBq) added to cultures containing early ring-stage parasites, which were allowed to develop in histidine-depleted culture medium for 30 hr. The cells were washed and thereafter solubilized in phosphate-buffered saline with 0.5% Triton X-100 and protease inhibitors. After centrifugation (Eppendorf centrifuge 5414) aliquots of the supernatant were added to protein A-Sepharose that had been preincubated with various antibody preparations. After washing, bound antigenic material was eluted by boiling in SDS-containing sample buffer and analyzed by SDS/PAGE and fluorography (Amplify, Amersham).

RESULTS

Characteristics of Rosettes. Spontaneous rosettes were stable cell aggregates that resisted repeated pipetting and centrifugation. The binding was temperature-independent (4°, 20°, 37°, and 41°C; data not shown) but sensitive to pH, favoring rosette formation at a slightly acidic pH (6.5–7.1; Fig. 2*A*). An abrupt drop in the rosetting rate was observed even at moderately alkaline pH (Fig. 2*A*). Sodium citrate dose-dependently and completely disrupted rosettes (Fig. 2*C*), whereas EDTA only gave inhibition up to 40% (Fig. 2*B*). The addition of Ca^{2+} or Mg^{2+} to divalent-cation-depleted cultured led to the rapid reappearance of rosettes (data not shown). As found previously (5), heparin strongly and dose-dependently reverted already existing rosettes (50% at 0.5–1.0 unit/ml; Fig. 2*D*).

Reversion of Rosettes by Antibodies. We investigated the role of various *P. falciparum* molecules in the formation of spontaneous rosettes. Both mono- and polyclonal antibodies [mouse mAb 89 (ascites) and rabbit antiserum MC1] to the histidine-rich protein PfHRP1/KP/KAHRP disrupted spontaneously formed rosettes in a dose-dependent manner (50% reversion at dilutions of 1:10 and 1:5, respectively; *P. falciparum* clone R^+PA1; Fig. 3) but did not inhibit endothelial cytoadherence (data not shown). Similar reversion of rosette formation by the anti-PfHRP1 antibodies was also obtained with a Thai isolate (R^+TM180). An immunoglobulin preparation containing mAb 89 separated from ascites on protein A-Sepharose gave 50% rosette reversion at 1.6 mg/ml. Serial dilutions of another 18 antisera or mAbs to *P. falciparum* antigens and one antiserum to a histidine-rich *P. lophurae* antigen (PlHRP) were also tested for the ability to abolish already formed rosettes with R^+PA1-infected erythocytes. However, none of the antibodies to these *P. falciparum* antigens had any effect on rosetting (Fig. 3), among them an antibody (human mAb 33G2) that inhibits adherence of infected erythrocytes to endothelial cells (19).

Immunofluorescence, Immunoblotting, and Immunoprecipitation. After acetone fixation, our rosetting parasites (R^+K^-PA1 and R^+K^+TM180) were stained in immunofluorescence assays by the anti-PfHRP1 antibodies (end-point titers 1:80) in a similar pattern (Fig. 4 *Left*) to that previously reported (16). No staining was seen with nonrosetting K^- strains (R^-FCR3; R^-F32). Other antibodies tested (anti-PfHRP2, anti-PfEMP2, and anti-Ag332) gave end-point titers between 1:320 and 1:2560 with both R^+ and R^- parasites, while the anti-PfHRP3 did not give any staining with these parasites. In immunoblotting, the anti-PfHRP1 antibodies also showed binding to a polypeptide of M_r 90,000 confined to R^+ strains (Fig. 4 *Center*). A distinct staining of the M_r 90,000 polypeptide was obtained only when preparations of enriched R^+ parasites (>50% parasitemia) were used. The rabbit serum MC1 recognized additional bands in the M_r 60,000–70,000 range but irrespective of rosetting phenotype probably representing crossreactivity with PfHRP2 (Fig. 4 *Center*). In immunoprecipitation, a M_r 28,000 polypeptide was precipitated with mAb 89 (anti-PfHRP1) from an extract of [^{3}H]histidine-labeled R^+ parasites (Fig. 4 *Right*). However, rabbit serum MC1 (anti-PfHRP1) did not react with any polypeptide in this assay.

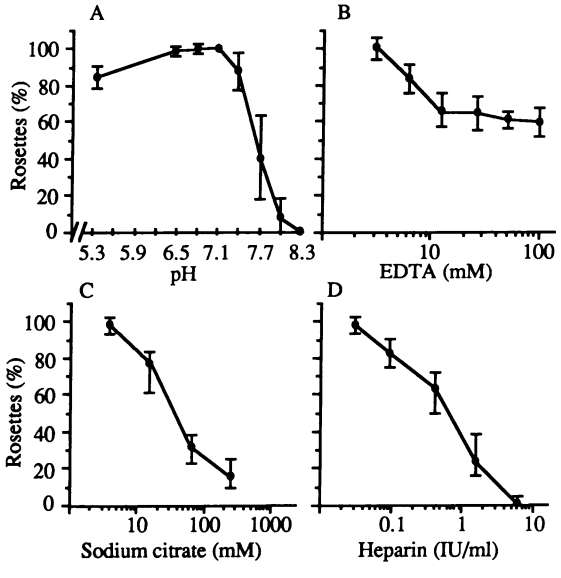

FIG. 2. pH dependence (*A*), and effect of EDTA (*B*), sodium citrate (*C*), or heparin (*D*) on the rosetting rate of *P. falciparum*-infected erythrocytes. The mean and range from at least two experiments are shown.

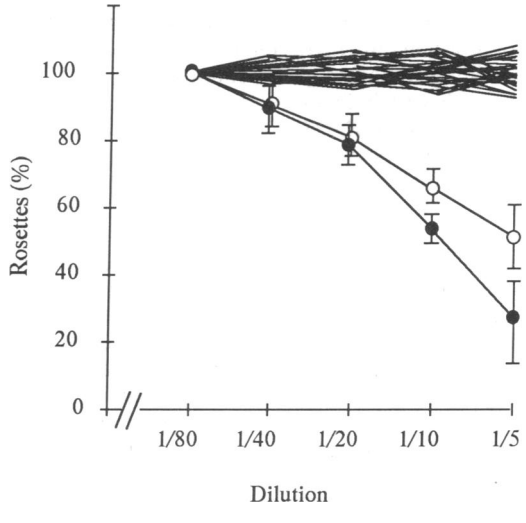

FIG. 3. Effect of serial dilutions of 20 antisera or mAbs to *P. falciparum* antigens and 1 antiserum to a *P. lophurae* antigen on rosetting rate. Anti-PfHRP1 mAb 89 (●) and rabbit serum MC1 (○) decreased the rate. All other sera tested (see text) are represented by the cluster of lines. For the anti-PfHRP1 antibodies, mean and range from at least three independent experiments are shown.

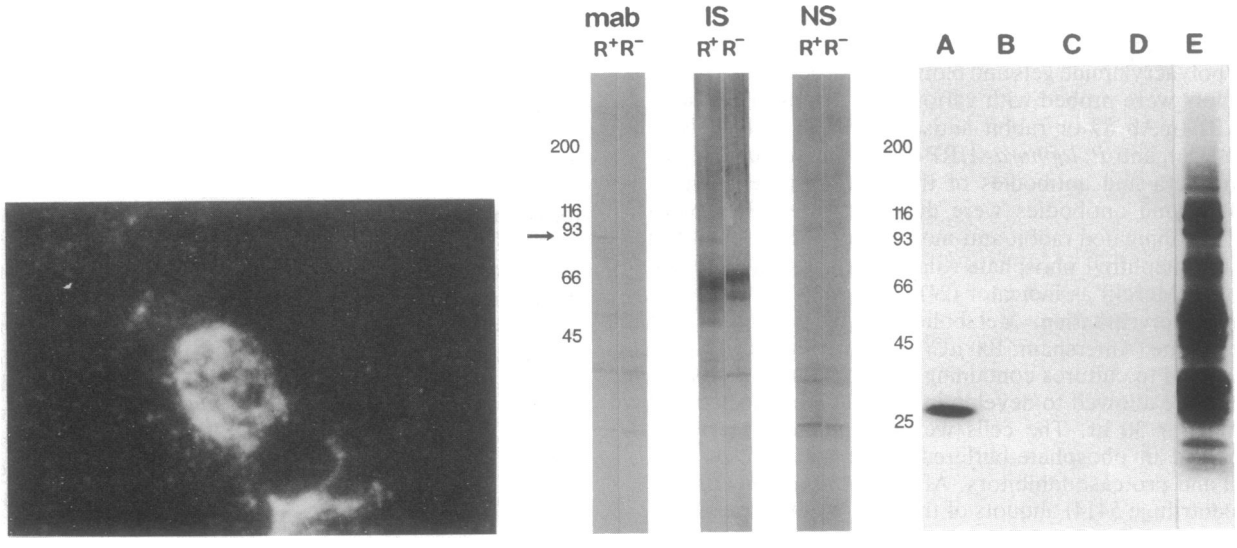

FIG. 4. (*Left*) Indirect immunofluorescence of acetone-fixed monolayers of rosetting infected erythrocytes with the anti-PfHRP1 mAb 89 (dilution 1:80). (*Center*) Immunoblot of infected erythrocytes with anti-PfHRP1 antibodies. mab, mAb 89; IS, rabbit serum MC1; NS, normal rabbit serum; R^+, Palo Alto (K^-R^+); R^-, F32 (K^-R^-). Numbers represent molecular weight ($M_r \times 10^{-3}$); arrow indicates M_r 90,000. (*Right*) Fluorography of metabolically labeled material immunoprecipitated from clone R^+PA1 with anti-PfHRP1 mAb 89 (lane A), anti-PfHRP1 rabbit serum MC1 (lane B), normal mouse serum (lane C), or normal rabbit serum (lane D). Lane E contained total Triton X-100-soluble material. Numbers represent molecular weight ($M_r \times 10^{-3}$).

DISCUSSION

An *in vitro* assay has been developed to investigate the molecular mechanisms behind spontaneous erythrocyte rosette formation in *P. falciparum* malaria. The assay is simple, rapid, and reproducible and measures the ability of different substances to disrupt already formed rosettes. The results so obtained may therefore be of clinical relevance, e.g., if antibodies are applied to dissolve aggregates of erythrocytes *in vivo*.

The binding between infected and uninfected erythrocytes was studied and found to be dependent on divalent cations (Ca^{2+}/Mg^{2+}) and sensitive to pH, with rosette formation favored at a slightly acidic pH as expected for the sites where sequestration and vessel occlusion occur. In addition, it was found to be sensitive to heparin, which disrupts rosettes at low concentrations (50% at 0.5–1.0 unit/ml) but to be independent of the ambient temperature. Spontaneous rosetting thus shares some important characteristics with other cell–cell recognition events involving molecules such as integrins or extracellular adhesive glycoproteins (25).

Amino acids with high ion-binding capacity (e.g., histidine) are common in some *P. falciparum* antigens associated with the infected erythrocyte membrane (10). Interestingly, there is also a histidine-rich glycoprotein in normal human plasma that partakes in coagulation homeostasis, interacts with metal ions or heparin, and mediates spontaneous rosette formation of thymocytes (26, 27). In the present study we investigated the role of various *P. falciparum* proteins in the formation of spontaneous rosettes. Both antisera and purified antibodies to one of the histidine-rich proteins (PfHRP1) disrupted the spontaneously formed rosettes in a dose-dependent manner (Fig. 3). Relatively high concentrations were needed to disrupt rosettes under the experimental conditions used. However, this was not unexpected, as rosetting probably involves several membrane interactions stabilizing the binding between the cells. In any event, the antibodies disrupted the rosetting seen with the laboratory strain R^+PA1 as well as with a recently isolated *P. falciparum* strain obtained from a patient with acute malaria (R^+TM180). However, no effects on rosetting were seen with antibodies to related histidine-rich proteins of *P. lophurae* and *P. falciparum*, suggesting that the reactive epitope(s) of the anti-PfHRP1 antibodies are located outside the histidine-rich and potentially crossreactive sequences of the molecules. Antibodies to other surface-associated *P. falciparum* antigens also failed to affect spontaneous rosetting, among them a human mAb (33G2) that inhibits adherence of infected erythrocytes to endothelial cells (19). The findings therefore indicate that rosetting and endothelial cytoadherence are mediated by different molecular mechanisms.

PfHRP1 was first discovered associated with *P. falciparum* parasites expressing knoblike protrusions on the surface of the infected erythrocytes (K^+ phenotype) (17, 28, 29) but has also been found in a knobless (K^-) strain (30). It is synthesized as a M_r 80,000–120,000 polypeptide early after merozoite invasion and exported to the membrane of cells infected with trophozoites and schizonts (28–30), stages that form spontaneous rosettes. The above results, which suggest the involvement of PfHRP1 in the rosetting process, are somewhat surprising since several previous studies suggested that this protein was confined to the submembranous part of knobs and not exposed on the erythrocyte surface (10, 16). Some authors, however, claim the existence of this protein on the cell surface, since it is cleaved by trypsin (31), an enzyme that also disrupts spontaneously formed rosettes (5, 12). After acetone fixation, our rosetting parasites were stained in immunofluorescence by the anti-PfHRP1 antibodies in a similar pattern to that previously reported, and in immunoblotting both mAb 89 and antiserum MC1 bound to a polypeptide of M_r 90,000 that was confined to R^+ strains. However, mAb 89, which has been shown to be specific for PfHRP1 (16), also immunoprecipitated a M_r 28,000 polypeptide from extracts of [^{3}H]histidine-labeled R^+ parasite. In addition, and as shown by others (17), antiserum MC1 does crossreact with the other histidine-rich proteins. Thus, although PfHRP1 seems to be the target of the antibodies, further studies are needed to identify directly the molecules that mediate rosetting. Furthermore, the presence of yet another crossreactive antigen cannot be excluded. The results obtained might alternatively be explained by the existence of different isoforms of PfHRP1 or by quantitative differences in expression of the molecule in R^+ and R^- strains. Moreover, it remains to be established whether the

reactive epitope constitutes the rosetting receptor or merely is located close to it.

In a study of erythrocyte sequestration (2), it was concluded that "cerebral malaria patients have a higher density of packing and considerably larger proportion of tightly packed vessels than falciparum patients with noncerebral disease." We have postulated that such aggregates of erythrocytes are partly due to spontaneous rosette formation, adding a new qualitative and quantitative dimension to endothelial cytoadherence (4, 5, 32). In support for this idea are the findings that anti-PfHRP1 antibodies revert already formed rosettes and that PfHRP1 is deposited in capillaries occluded by erythrocytes in patients with cerebral malaria (33). Furthermore, heparin, which disrupts rosettes but has almost no effect on *in vitro* endothelial cytoadherence, may also have a beneficial effect on the clinical outcome of cerebral malaria (34). These observations, in addition to the results reported here, support the hypothesis of two separate mechanisms in the pathogenesis of cerebral malaria: endothelial adhesion and spontaneous rosetting.

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1. Aikawa, M. (1988) *Am. J. Trop. Med. Hyg.* **39,** 3–10.
2. MacPherson, G. G., Warrell, M. J., White, N. J., Looareesuwan, S. & Warrell, D. A. (1985) *Am. J. Pathol.* **119,** 385–401.
3. Oo, M. M., Aikawa, M., Than, T., Aye, T. M., Myint, P. T., Igarashi, I. & Schoene, W. C. (1987) *J. Neuropathol. Exp. Neurol.* **46,** 223–231.
4. Wahlgren, M. (1986) Ph.D. Thesis (Karolinska Institutet, Stockholm).
5. Udomsangpetch, R., Wåhlin, B., Carlson, J., Berzins, K., Torii, M., Aikawa, M., Perlmann, P. & Wahlgren, M. (1989) *J. Exp. Med.* **169,** 1835–1840.
6. Trager, W., Rudzinska, M. A. & Bradbury, P. C. (1968) *Bull. W. H. O.* **35,** 883–885.
7. Luse, S. A. & Miller, L. H. (1971) *Am. J. Trop. Med. Hyg.* **20,** 655–660.
8. Raventos-Suarez, C., Kaul, D. K., Macaluso, F. & Nagel, R. L. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3829–3833.
9. Smalley, M. E., Abdalla, S. & Brown, J. (1980) *Trans. R. Soc. Trop. Med. Hyg.* **75,** 103–105.
10. Howard, R. J. (1988) *Prog. Allergy* **41,** 98–147.
11. Biggs, B. A., Culvenor, J. G., Ng, J. S., Kemp, D. J. & Brown, G. V. (1989) *Exp. Parasitol.* **69,** 189–197.
12. Handunnetti, S. M., David, P. H., Perera, K. L. R. L. & Mendis, K. N. (1989) *Am. J. Trop. Med. Hyg.* **40,** 115–118.
13. David, P. H., Handunnetti, S. M., Leech, J. H., Gamage, P. & Mendis, K. N. (1988) *Am. J. Trop. Med. Hyg.* **38,** 289–297.
14. David, P. H., Hommel, M., Miller, L. H., Udeinya, I. J. & Oligino, L. D. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5075–5079.
15. Trager, W. & Jensen, J. B. (1976) *Science* **193,** 673–675.
16. Taylor, D. W., Parra, M., Chapman, G. B., Stearna, M. E., Rener, J., Aikawa, M., Uni, S., Aley, S. B., Panton, L. J. & Howard, R. J. (1987) *Mol. Biochem. Parasitol.* **25,** 165–174.
17. Rock, E. P., Saul, A. J., Taylor, D. W., Leech, J. H., Sherwood, J. A. & Howard, R. J. (1988) *Infect. Immun.* **56,** 3301–3304.
18. Berzins, K., Perlmann, H., Wåhlin, B., Carlsson, J., Wahlgren, M., Udomsangpetch, R., Björkman, A., Patarroyo, M. E. & Perlmann, P. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1065–1069.
19. Udomsangpetch, R., Aikawa, M., Berzins, K., Wahlgren, M. & Perlmann, P. (1989) *Nature (London)* **338,** 763–765.
20. Mattei, D., Berzins, K., Wahlgren, M., Udomsangpetch, R., Perlmann, P., Griesser, H. W., Scherf, A., Müller-Hill, B., Bonnefoy, S., Guillotte, M., Langsley, G., Pereira da Silva, L. & Mercereau-Puijalon, O. (1989) *Parasite Immunol.* **11,** 15–30.
21. Anders, R. F., Coppel, R. L., Brown, G. V. & Kemp, D. J. (1988) *Prog. Allergy* **41,** 148–172.
22. Wahlgren, M., Åslund, L., Franzén, L., Sundvall, M., Wåhlin, B., Berzins, K., McNicol, L. A., Björkman, A., Wigzell, H., Perlmann, P. & Pettersson, U. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2677–2681.
23. Robson, K. J. H., Hall, J. R. S., Jennings, M. W., Harris, T. J. R., Marsh, K., Newbold, C. I., Tate, V. E. & Weatherall, D. J. (1988) *Nature (London)* **335,** 79–82.
24. Holmquist, G., Udomsangpetch, R., Berzins, K., Wigzell, H. & Perlmann, P. (1988) *Infect. Immun.* **56,** 1545–1550.
25. Hemler, M. E. (1988) *Immunol. Today* **9,** 109–113.
26. Lijnen, H. R., Hoylaerts, M. & Collen, D. (1983) *J. Biol. Chem.* **258,** 3803–3808.
27. Morgan, W. T. (1978) *Biochem. Biophys. Acta* **533,** 319–333.
28. Kilejian, A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4650–4653.
29. Hadley, T. J., Leech, J. H., Green, T. J., Daniel, W. A., Wahlgren, M., Miller, L. H. & Howard, R. J. (1983) *Mol. Biochem. Parasitol.* **9,** 271–278.
30. Rock, E. P., Marsh, K., Saul, A. J., Wellems, T. E., Taylor, D. W., Maloy, W. L. & Howard, R. J. (1987) *Parasitology* **95,** 209–227.
31. Vernot-Hernandez, J. P. & Heidrich, H. G. (1985) *Z. Parasitenkd.* **71,** 41–51.
32. Wahlgren, M., Carlson, J., Udomsangpetch, R. & Perlmann, P. (1989) *Parasitol. Today* **5,** 183–185.
33. Igarishi, I., Oo, M. M., Stanley, H., Reese, R. & Aikawa, M. (1987) *Am. J. Trop. Med. Hyg.* **37,** 511–515.
34. Smitskamp, H. & Wolthuis, F. H. (1971) *Br. Med. J.* **1,** 714–716.