cycloheximide or released with 2 mM puromycin in buffer A. Ribosome-bound actin-ΔC was derived from RNAs transcribed from pGEM–mouse β-actin linearized with Apa I (amino acids 1 to 368), Xba I (amino acids 1 to 220), or Sna BI (amino acids 1 to 133). Coimmunoprecipitation with chaperones was performed as in (*8*). Truncated actin chains remained unfolded, as indicated by their sensitivity toward proteinase K and by their inability to bind to DNase I. Similar results were obtained with each truncation in the degradation assay.

18. The addition of T-GroEL at 1 and 3 μM reduced the efficiency of translation by ~30%, which explains the decrease in the amount of native actin seen in Fig. 3D. Quantification of the amount of full-length actin in these translations indicated that there was no decrease in the fraction of folded actin (see Fig. 3A). Addition of T-GroEL reduced the amount of TRiC-bound actin chains because the translation reaction contains incomplete chains that are unable to fold. In contrast to T-GroEL, addition of T-TRiC [see (*25*) and Fig. 4B] inhibited the folding of actin when added from the beginning of translation.
19. Ribosomes were isolated by sedimentation through a sucrose solution as described (*8*). A 50-μl actin translation reaction was diluted threefold in buffer B [buffer A containing 5 mM $Mg(AcO)_2$, 0.5 mM cycloheximide, 1 mM DTT, RNAsin (Promega, 1 U/μl), 1 mM phenylmethylsulfonyl fluoride, aprotinin (2 μg/ml), and leupeptin (0.5 μg/ml)], and ATP was removed by incubation with apyrase (0.5 U/μl for 5 min at 25°C). The reaction was centrifuged for 10 min at 16,000*g* to remove aggregates and placed over 350 μl of 30% sucrose in buffer B. Ribosomal pellets obtained by centrifugation for 12 min at 120,000 rpm in a TL-120.1 rotor at 4°C were washed twice in buffer B, resuspended in the initial volume of buffer C [20 mM Hepes-KOH (pH 7.4), 100 mM KAcO, 5 mM $Mg(AcO)_2$, 10% glycerol, 5% polyethylene glycol, and 1 mM DTT] and centrifuged (16,000*g*, 10 min) to remove insoluble material.
20. CAT assays were performed as described [J. P. Hendrick, T. Langer, T. A. Davis, F. U. Hartl, M. Wiedmann, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10216 (1993)]. Native purified CAT (Boehringer) was used as a control; pGEM-CAT was provided by M. Wiedmann. Specific activities were calculated as the ratio of the measured enzymatic activity to the amount of full-length ^{35}S-labeled protein as determined by SDS-PAGE and Phosphorimager analysis. Native PAGE of CAT translation reactions resolved TRiC-bound chains from assembled CAT trimers. The decrease in CAT activity upon addition of T-GroEL was accompanied by an increase in the amount of newly translated full-length CAT bound to T-GroEL. Transfer from TRiC to T-GroEL was also observed for newly translated α-tubulin. This protein can fold on TRiC into its GTP-binding conformation (*30*), but before the formation of tubulin dimers exposes hydrophobic surfaces that may allow it to cycle on and off the chaperonin. Thus, the exclusion of T-GroEL from productive nascent chains of actin and luciferase cannot be explained by an intrinsic lack of binding capacity of T-GroEL for newly translated polypeptide.
21. Hemin is essential for translation in reticulocytes but inhibits proteasome function [A. L. Haas and I. A. Rose, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6845 (1981)]; we removed it by desalting the lysates containing ribosome-associated actin-ΔC on Sephadex G-25 columns equilibrated in buffer A. Actin-ΔC was degraded through the proteasome-ubiquitin pathway, because degradation was inhibited by hemin (with the accumulation of ubiquitin conjugates) and by methylated ubiquitin [A. Hershko, D. Ganoth, J. Pehrson, R. E. Palazzo, L. H. Cohen, *J. Biol. Chem.* **266**, 16376 (1991)]. Similar results were obtained for actin-ΔC 1–220 and actin-ΔC 1–368.
22. The protection of nascent actin chains against degradation was underestimated because of the spontaneous release of nascent chains from the ribosome at 37°C (Fig. 3E), which we measured by including hemin during the incubation with cycloheximide and separating ribosome-bound and released chains by sedimentation through a dense sucrose solution (*19*).
23. Release of the chaperone as a requirement for degradation was also observed for Hsp70-bound polypeptides in mitochondria [I. Wagner, H. Arlt, L. van Dyck, T. Langer, W. Neupert, *EMBO J.* **13**, 5135 (1994)].
24. Evidence has been presented that during TRiC-mediated folding, actin undergoes cycles of ATP-dependent release and rebinding involving its discharge into the solution in a non-native state [G. Tian, I. E. Vainberg, W. D. Tap, S. A. Lewis, N. J. Cowan, *Nature* **375**, 250 (1995)].
25. TRiC was purified from bovine testes as in (*5*). ^{35}S-labeled D-actin was diluted 100-fold into 0.2 μM TRiC in buffer C (*19*). After incubation for 5 min at 30°C, aggregates were removed by centrifugation. Unbound actin was separated from TRiC-actin complexes by chromatography on a Mono-Q HR5/5 column with a gradient from 100 to 400 mM KAcO in buffer D [20 mM Hepes-KOH (pH 7.4), 100 mM KAcO, 5 mM $Mg(AcO)_2$, 10% glycerol, and 1 mM DTT]. T-TRiC was prepared by incubating TRiC in buffer D with 2 mM 8-azido-ATP in the dark for 5 min at 30°C, followed by crosslinking [by exposure to UV light (310 to 340 nm) for 10 min at 4°C] and repurification. Folding reactions were started by addition of 1 mM ATP and were incubated for 40 min at 30°C.
26. We confirmed the previous observation that actin can be released from TRiC in a non-native state (*24*) with a diluted actin-TRiC complex (25 nM) under conditions where we found bovine TRiC to be structurally labile. The lack of inhibition of actin folding by T-GroEL observed with native PAGE (Fig. 4A) was confirmed by the DNase I binding assay.
27. The crosslinked form (T-TRiC) displayed a faster mobility on native PAGE than did TRiC (Fig. 4B), either because of the added negative charge conferred by the covalently bound nucleotide or because of a conformational change triggered by nucleotide binding.
28. Identical results were obtained for T-GroEL and T-TRiC when actin folding was analyzed as a function of binding to DNase I–Sepharose beads.
29. B. Wiedmann, H. Sakai, T. A. Davis, M. Wiedmann, *Nature* **370**, 434 (1994); S. Wang, H. Sakai, M. Wiedmann, *J. Cell Biol.* **130**, 519 (1995).
30. G. L. Tian, I. E. Vainberg, W. D. Tap, S. A. Lewis, N. J. Cowan, *J. Biol. Chem.* **270**, 23910 (1995).
31. J. M. Solomon, J. M. Rossi, K. Golic, T. McGarry, S. Lindquist, *New Biol.* **3**, 1106 (1991); B. Freeman and R. Morimoto, *EMBO J.* **12**, 2969 (1996); D. A. Parsell, A. S. Kowal, M. A. Singer, S. Lindquist, *Nature* **372**, 475 (1994).

32. We thank M. Wiedmann for pGEM-CAT, J. Shephard for pGEM–mouse β-actin, and M. Mayhew, D. McColl, and B. Netzer for critical reading of the manuscript. Supported by NIH and the Howard Hughes Medical Institute.

13 March 1996; accepted 22 April 1996

Adherence of *Plasmodium falciparum* to Chondroitin Sulfate A in the Human Placenta

Michal Fried and Patrick E. Duffy

Women are particularly susceptible to malaria during first and second pregnancies, even though they may have developed immunity over years of residence in endemic areas. *Plasmodium falciparum*–infected red blood cells (IRBCs) were obtained from human placentas. These IRBCs bound to purified chondroitin sulfate A (CSA) but not to other extracellular matrix proteins or to other known IRBC receptors. IRBCs from nonpregnant donors did not bind to CSA. Placental IRBCs adhered to sections of fresh-frozen human placenta with an anatomic distribution similar to that of naturally infected placentas, and this adhesion was competitively inhibited by purified CSA. Thus, adhesion to CSA appears to select for a subpopulation of parasites that causes maternal malaria.

Falciparum malaria can present with various clinical manifestations, including coma, pulmonary edema, renal insufficiency, and severe anemia. The molecular basis for particular syndromes is unknown, although IRBC cytoadherence is generally considered to contribute to the development of cerebral malaria (*1*), and some authors have suggested that virulent strains (*2*) or "virulence factors" (*3*) of *P. falciparum* cause severe disease or death. Maternal malaria, a distinct clinical entity, is a major cause of pregnancy-related complications in endemic areas; it is associated with premature delivery, intrauterine growth retardation, perinatal mortality in the infant, and anemia and death in the mother (*4*, *5*).

After years of exposure to malaria, women have acquired immunity to *P. falciparum* equivalent to their male counterparts (*6*). Pregnant women, however, have a unique susceptibility to malaria infection, which diminishes as their gravid status increases (*5*). In the absence of evidence for other mechanisms, maternal malaria has been attributed to the immunosuppression of pregnancy (*7*), which is also thought to lead to increased frequency of certain viral infections such as rubella (*8*). Because susceptibility to infection is markedly lower in multigravid women than in primigravid women, immunosuppression cannot entirely explain the phenomenon (*9*).

The placenta is a preferential site for sequestration of IRBCs and can experience high parasite densities while the peripheral circulation is free of parasites (*6*, *10*). In other vascular beds, IRBCs adhere to the endothelial surface; endothelial surface molecules such as thrombospondin, CD36, intercellular adhesion molecule–1 (ICAM-1),

U.S. Army Medical Research Unit–Kenya and Kenya Medical Research Institute, Post Office Box 54, Kisumu, Kenya.

vascular cell adhesion molecule–1 (VCAM-1), and E-selectin are known to support IRBC adhesion in vitro (*11*). A glycosaminoglycan, chondroitin sulfate A (CSA), was recently identified as a receptor for IRBCs (*12, 13*).

IRBCs obtained directly from infected human placentas (*14*) were examined in assays that measured adhesion (*11*) to immobilized extracellular matrix proteins. Relative to the number of IRBCs bound to bovine serum albumin (BSA), the number adhering to CSA (Fig. 1A) was significantly elevated (P = 0.0003, Mann-Whitney U); the mean number of cells bound to CSA was 22 times the number bound to BSA. No other extracellular matrix protein tested—including chondroitin sulfate B (CSB), fibrinogen, cellular and plasma fibronectin, fucoidin, collagens III, IV, VI, VIII, IX, and X, and laminin—was able to support binding exceeding that of BSA. Five isolates were also assayed for adhesion to chondroitin sulfate C (CSC) and heparan sulfate glycoprotein; the number of IRBCs bound to these molecules was no greater than the number bound to BSA. In competition assays (Fig. 1B), adhesion to CSA was inhibited by preincubation of IRBCs with CSA in solution (median inhibitory concentration IC_{50} = 1.2 μg/ml). Preincubation with CSB, which contains about 10% CSA in the form provided by its supplier, inhibited adhesion only at a much higher concentration (IC_{50} = 300 μg/ml), which suggested that the CSA fraction may be responsible for reducing adhesion.

To examine the pattern of adhesion in tissue, we incubated isolates of placental IRBCs on sections of fresh-frozen, uninfected human placenta for 1 hour, then gently washed away unbound cells. Placental IRBCs bound preferentially to the trophoblastic villi, extracellular villi, and syncytial bridges (Fig. 2, A and B) in a pattern similar to the in vivo localization of IRBCs observed in sections of naturally infected placentas. IRBC adhesion to tissue sections was inhibited in the presence of CSA but not in the presence of collagen VI. IRBC binding was also inhibited in the presence of the CSB preparation, but the IC_{50} value was about 12 times that of CSA, which again suggested that the CSA fraction of the CSB product was responsible for abrogating adhesion (Fig. 2C). Adhesion was inhibited by pretreatment of the tissue section with chondroitinase AC (*15*), a polysaccharide lyase derived from *Flavobacterium heparinum* that acts endolytically on CSA, CSC, and hyaluronate. Chondroitinase AC does not act on heparin, heparan sulfate, or keratan sulfate (*16*). Enzymatic digestion reduced adhesion to less than 5% of that supported by untreated tissue and also abolished immunostaining of placenta sections with a monoclonal antibody to CSA and CSC; immunostaining with antibodies to collagen IV or to laminin was not affected by such treatment (*17*).

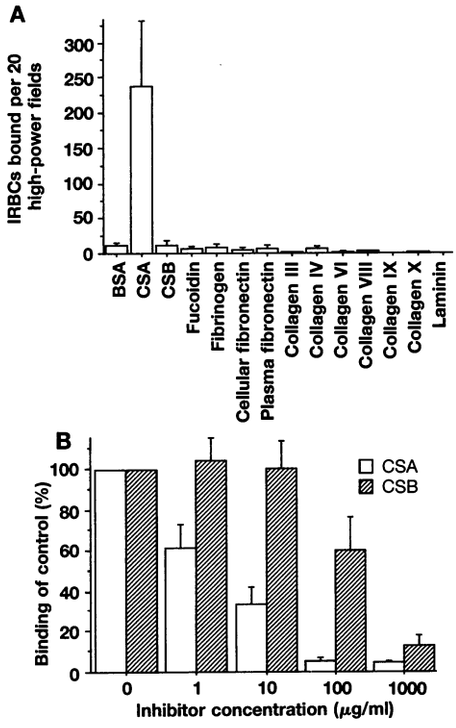

Fig. 1. Binding of placental parasites to extracellular matrix proteins. (**A**) Extracellular matrix protein in solution (10 μg/ml) was adsorbed on a petri dish and then overlaid with an IRBC suspension of 5 to 20% parasitemia at 5% hematocrit. After incubation at 37°C for 1 hour, unbound cells were washed off with PBS, and bound IRBCs were fixed with 2% glutaraldehyde. Values are presented as mean numbers of parasites counted per 20 high-power fields (magnification, ×1000) from assays of 11 different placental isolates. (**B**) Competition assay of placental IRBC binding to immobilized CSA. Placental parasites were preincubated with increasing concentrations of CSA or CSB for 1 hour at 37°C, after which the binding assay was performed as above. Values are presented as the mean percent binding of control obtained from seven independent isolates. Binding of isolates in the absence of soluble competitor ranged from 83 to 705 IRBCs per 20 high-power fields. Error bars represent SE.

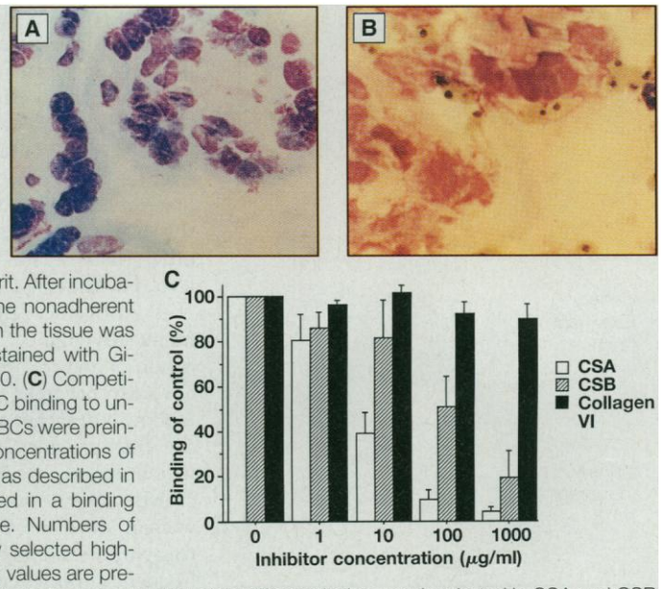

Fig. 2. Adhesion of placental IRBCs to uninfected placenta tissue. (**A**) Uninfected placenta tissue (fresh-frozen, Giemsa-stained). (**B**) Binding of placental IRBCs to uninfected placenta tissue. A section of uninfected placenta tissue was overlaid with IRBCs suspended at 5% hematocrit. After incubation for 1 hour at 37°C, the nonadherent cells were washed off, then the tissue was fixed with methanol and stained with Giemsa. Magnifications, ×400. (**C**) Competition assay of placental IRBC binding to uninfected placenta tissue. IRBCs were preincubated with increasing concentrations of CSA, CSB, or collagen VI, as described in Fig. 1, and were then used in a binding assay as described above. Numbers of parasites per 20 randomly selected high-power fields were counted; values are presented as the mean percent binding of control for eight different isolates preincubated in CSA and CSB and for two isolates preincubated in collagen VI. Binding of isolates in the absence of soluble competitor ranged from 36 to 2561 IRBCs per 20 high-power fields.

These observations suggested that a distinct subpopulation of parasites is responsible for maternal malaria. We compared the binding characteristics of IRBCs obtained from the peripheral circulation of nonpregnant donors with those of IRBCs from the placenta (Table 1). In these assays, placental IRBCs bound in substantial numbers to CSA but not to CD36. Conversely, peripheral parasites from nonpregnant hosts adhered to CD36 but not to CSA, which supports the hypothesis that specific malaria syndromes (in this case, placental malaria) are caused by distinct *P. falciparum* subpopulations. Parasites obtained from the peripheral circulation of pregnant hosts can have a CD36-

binding phenotype, a CSA-binding phenotype, or a mixed phenotype (Table 1). Several isolates drawn from the placenta, the periphery of pregnant hosts, and the periphery of nonpregnant hosts (seven, five, and four isolates, respectively) were assayed on ICAM-1; none demonstrated adhesion (*17*).

The particular susceptibility of primigravid and secundigravid women to severe malaria has been a vexing problem with enormous clinical consequences (*4*, *5*). Maternal parasitemia rates as high as 65% have been observed among primigravid women in endemic areas (*6*), with a peak prevalence between 9 and 16 weeks gestation (*18*). Our adhesion studies suggest that maternal malaria arises when the placenta selects for a parasite subpopulation that binds CSA. Glycosaminoglycans are also used as receptors for binding or invasion by several other microorganisms, including *P. yoelii* (*19*), *Trypanosoma cruzi* (*20*), and *Varicella zoster* (*21*).

Table 1. Binding phenotype of placental parasites versus peripheral parasites. Binding to immobilized CSA, CD36, and BSA was performed as described in Fig. 1. Peripheral parasites were kept overnight in culture to allow parasites to develop into mature forms. All parasite samples were concentrated on a gelatin gradient (except placental sample K). Placental parasites and peripheral parasites from pregnant women with the same sample code refer to samples obtained from the same donor. The results are expressed as numbers of parasites counted per 20 high-power fields. NT, not tested.

Parasite source	Sample code	Binding substrate		
		BSA	CSA	CD36
Placenta (pregnant host)	A	56	894	NT
	B	2	69	NT
	C	1	118	11
	D	0	71	3
	E	0	32	2
	F	0	207	3
	G	0	207	3
	H	2	110	2
	I	1	103	3
	J	15	79	4
	K	0	68	3
	L	0	195	10
	M	1	1354	12
	N	1	43	2
Periphery (pregnant host)	A	29	29	NT
	B	0	0	NT
	C	0	0	32
	D	0	0	20
	E	1	75	3
	F	9	19	27
	G	5	164	15
Periphery (nonpregnant host)	a	1	0	41
	b	20	16	461
	c	3	2	NT
	d	1	0	59
	e	4	9	143

In earlier studies, the vast majority of *P. falciparum* field isolates adhered to CD36 (*22*), whereas a minority of isolates contained smaller numbers of IRBCs that bound to CSA (*12*). IRBCs obtained from the placenta consistently bind to CSA but not to other receptors. IRBCs drawn from the peripheral circulation of pregnant women can bind to CD36, CSA, or both. These findings suggest that parasites seen on the blood smear of a pregnant woman may be CSA-binding parasites derived from the placenta, may be CD36-binding parasites circulating outside the placenta, or may be a mixture of the two subpopulations. Corollary to this observation, IRBCs obtained from the peripheral circulation of malarious individuals may or may not represent the parasite subpopulation that elicits particular symptoms; this ambiguity confounds attempts to correlate parasite phenotypes with clinical syndromes.

We propose a model for maternal malaria whereby a parasite subpopulation infrequently occurring in nonpregnant hosts preferentially sequesters and multiplies in the placenta. These parasites differ from other field isolates in their adhesion pattern, but they also may have alterations in other antigenic determinants to evade immune surveillance. Despite humoral recognition of an array of parasite antigens (*23*), a woman becomes highly susceptible to infection during her initial pregnancy, when she first provides the placental substrate, which selects CSA-binding parasites for growth. With successive pregnancies, a woman develops increasing immunity to this parasite subpopulation and reduces her frequency and severity of malaria infection.

REFERENCES AND NOTES

1. M. Riganti *et al.*, *Immunol. Lett.* **25**, 199 (1990).
2. S. Gupta *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3715 (1994).
3. P. Rindwald *et al.*, *Infect. Immun.* **61**, 5198 (1993); K. P. Day *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 8292 (1993).
4. C. Menendez, *Parasitol. Today* **11**, 178 (1995).
5. I. A. McGregor, *Parassitologia* **29**, 153 (1987).
6. B. J. Brabin, *Bull. WHO* **61**, 1005 (1983).
7. E. D. Weinberg, *Rev. Infect. Dis.* **6**, 814 (1984); B. J. Brabin, *ibid.* **7**, 579 (1985).
8. K. Reilly and N. Clemenson, *Prim. Care* **20**, 665 (1993).
9. I. A. McGregor, M. E. Wilson, W. Z. Dillewicz, *Trans. R. Soc. Trop. Med. Hyg.* **77**, 232 (1983).
10. P. R. Walter, Y. Garin, P. Blot, *Am. J. Pathol.* **109**, 330 (1982).
11. J. W. Barnwell, C. F. Ockenhouse, D. M. Knowles, *J. Immunol.* **135**, 3494 (1985); C. F. Ockenhouse, R. Betageri, T. A. Springer, D. E. Staunton, *Cell* **68**, 63 (1992); C. F. Ockenhouse *et al.*, *J. Exp. Med.* **176**, 1183 (1992); D. D. Roberts *et al.*, *Nature* **318**, 64 (1985).
12. S. J. Rogerson *et al.*, *J. Exp. Med.* **182**, 15 (1995).
13. C. Robert *et al.*, *Res. Immunol.* **146**, 383 (1995).
14. Women aged 18 and above who were admitted to deliver at the New Nyanza Provincial Hospital, Kisumu, Kenya, were asked to participate in the study. All volunteers signed an informed consent form translated into their native language after receiving a study explanation form and an oral explanation from a nurse in their native language.
15. Uninfected placenta tissue was incubated with 0.1 U of chondroitinase AC for 30 min at 37°C, after which the tissue was rinsed several times with phosphate-buffered saline (PBS), and a binding assay was performed as described in Fig. 2, A and B.
16. F. M. Ausubel *et al.*, *Current Protocols in Molecular Biology* (Wiley, New York, 1993).
17. M. Fried and P. E. Duffy, data not shown.
18. B. J. Brabin *et al.*, *Ann. Trop. Med. Parasitol.* **84**, 1 (1990).
19. S. J. Pancake, G. D. Holt, S. Mellouk, S. L. Hoffman, *J. Cell Biol.* **117**, 1351 (1992).
20. E. M. Herrera, M. Ming, E. Ortega-Barria, M. E. A. Pereira, *Mol. Biochem. Parasitol.* **65**, 73 (1994).
21. Z. Zhu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 3546 (1995).
22. C. F. Ockenhouse *et al.*, *J. Infect. Dis.* **164**, 163 (1991).
23. F. N. Rasheed *et al.*, *Am. J. Trop. Med. Hyg.* **48**, 154 (1993).

24. We thank the Kenya Medical Research Institute and D. Gordon for their support, R. Muga and H. Odollo for organizing sample collection, L. Miller and G. Ward for reviewing the manuscript, and R. Rothlein for ICAM-1. Supported in part by the Defense Women's Health Research Program. M.F. is a National Research Council fellow. The views expressed in this report do not necessarily reflect those of the U.S. Department of Defense.

11 December 1995; accepted 11 April 1996