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Cytoadherence Characteristics of Rosette-Forming *Plasmodium falciparum*

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Sequestration of *Plasmodium falciparum*-infected erythrocytes to the capillary endothelium can cause obstruction and localized tissue damage. Occlusion of vessels in falciparum malaria infection has been related to two properties of the parasite: adhesion to endothelial cells and rosette formation. Our study on *P. falciparum* isolates from Thailand producing variable numbers of rosettes suggests the involvement of rosettes in capillary blockage caused by direct adhesion of the rosette-forming infected erythrocytes to various target cells, e.g., live human umbilical vein endothelial cells, monocytes, and platelets. These rosettes did not bind Formalin-fixed target cells, nor did they bind to live or fixed C32 or G361 melanoma cells. Classification of the receptors involved in cytoadherence of endothelial cells and monocytes by specific antibody blocking and flow cytometry indicated that CD36 was involved in the adherence of monocytes but that other receptors besides CD36 may be involved in parasite adherence to endothelial cells. The cytoadherence of infected erythrocytes to monocytes was also associated with CD54 (ICAM-1). Further, differentiation of adherent monocytes resulted in an inversion of CD36 and CD54 levels on the cell surface which correlated with a decrease in surface binding of infected erythrocytes. This observation suggests that the state of cell activation and differentiation may also contribute to sequestration of parasites and to the pathogenesis of malaria.

Plasmodium falciparum may cause severe infection with high morbidity and mortality in both human and primate hosts (12, 13). The precise mechanisms are unclear, but occlusion of blood vessels by *P. falciparum*-infected erythrocytes (RBC), especially in the brain, results in pathophysiological changes (5, 28, 29), and delay in treatment may be fatal. The mechanism(s) of vascular occlusion by infected RBC appears to be related to the property of *P. falciparum* cytoadherence to endothelium in postcapillary venules (1, 17). Recently, an ex vivo model showing vascular obstruction by rosette-forming *P. falciparum*-infected RBC was described (10). Rosette formation, therefore, could be involved in sequestration-related obstruction in the host microvasculature and contribute to the pathogenesis of severe malaria. However, the importance of rosette-forming infected RBC in severe malaria in humans remains to be established. Studies on the cytoadherence of *P. falciparum*-infected RBC have involved a number of target cells derived from various human tissues, including umbilical vein endothelial cells (26), amelanotic melanoma cells (22), monocytes (3), platelets (15, 16), and erythrocytes (6, 27), that interact with infected RBC.

The mechanisms of binding to these target cells have been shown to involve a group of receptors on target cells and several parasite-derived protein ligands on the membrane of infected RBC (8). Receptors that have been reported previously include CD36 (2, 3, 14, 18, 19), intercellular adhesion molecule 1 (ICAM-1; also called CD54) (4), and thrombospondin (20, 21). A recent study demonstrated that the interaction between human infected RBC and normal RBC, rosette formation, is different from the interaction between infected RBC and melanoma cells (27), suggesting the presence of a distinct parasite ligand(s) or patterns of ligand-receptor interactions.

In this report, we describe the cytoadherence characteristics of two rosette-forming *P. falciparum* isolates and a nonrosetting isolate with various target cells. The rosetting *P. falciparum* parasites bound readily to fresh endothelial cells and monocytes both as rosettes and as individual infected RBC but did not bind to melanoma cells. The nonrosetting parasite did bind to the melanoma cell lines. Receptors involved in the binding of these infected RBC were investigated. Correlations between expression of markers on target cells and the binding efficiency of each target cell were assessed. Our observations support a role for CD36 as a receptor for infected RBC binding to human monocytes but not endothelial cells and suggest the presence of as yet unidentified receptors on endothelial cells that may contribute to the pathophysiological consequences of parasite sequestration in malaria.

MATERIALS AND METHODS

Antibodies and reagent. Monoclonal antibody (MAb) to CD36 (OKM5; immunoglobulin G1 [IgG1]) was purchased from Ortho Pharmaceuticals, Raritan, N.J.; MAb to ICAM-1 (BMS101, IgG2a) was from Bender Med System, Vienna, Austria; MAb to ICAM-1 (LB-2, IgG2b), CD14, and HLA-DR and mouse IgG1 and IgG2a MAb were from Becton Dickinson Immunocytometry Systems; and goat antiserum to human factor 8 was from ICN Biomedicals Inc., Costa Mesa, Calif. The $F(ab')_2$ fraction of rabbit anti-mouse or anti-goat immunoglobulin conjugated with fluorescein isothiocyanate (FITC) was obtained from Dako-

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patts a/s. Purified soluble CD36 was a gift from C. Ockenhouse, Washington, D.C.

Parasites. The Thai *P. falciparum* isolates TM178R and TM267R from Chantaburi and ND9 from Prajeenburi were maintained as previously described (24) with human group O RBC. Cells were cultured in medium RPMI 1640 (Seromed, Biochrom, Germany) supplemented with 2 mM glutamine, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 10% human serum, and 20 μg of gentamicin per ml (pH 7.4) at 37°C in a 5% CO_2 atmosphere. Cultures were maintained at 15 to 20% parasitized RBC (PRBC) and 3% hematocrit (Hct) in order to perform cytoadherence assays without agitation and yet obtain appropriate contact between target cells and PRBC.

Enumeration of rosette formation. One drop of *P. falciparum* culture mounted with a coverslip on a glass slide was examined by light microscopy. Rosette formation was characterized by a PRBC associated with two or more uninfected RBC. The number of rosettes in 500 PRBC containing malarial pigment was counted and calculated as a percentage of the rosettes in the culture.

Target cell preparation. Human umbilical vein endothelial cells were isolated and maintained in culture as described previously (9) and used from passages 2 through 4. For identification of endothelial cells, the cells from passage 3 were stained with antiserum to human factor 8 and FITC conjugate and analyzed by flow cytometry. The result showed that >99% of these cells were of the factor 8-positive phenotype. The cells were kept in M199 medium (Gibco Limited, Paisley, Scotland) supplemented with 200 μg of endothelial cell growth factor (Biochemical Supplies Co. Ltd., Bethesda, Mass.) per ml, 2 mM glutamine, 20% fetal calf serum, and 20 μg of gentamicin per ml (pH 7.4) at 37°C in a 5% CO_2 atmosphere. The endothelial cells were passaged at 80% confluence by treatment with 20 mM EDTA in phosphate-buffered saline (PBS), collection in suspension, and washing in medium. The cells were then resuspended with culture medium and grown to 2×10^4 cells per coverslip for 18 to 20 h.

Two melanoma cell lines, C32 and G361, were maintained in RPMI 1640 medium supplemented with 2 mM glutamine, 10% fetal calf serum, and 20 μg of gentamicin per ml (pH 7.4) at 37°C in a 5% CO_2 atmosphere. These cell lines were grown to 2×10^5 cells per coverslip for 18 to 20 h.

Mononuclear cells were separated by overlayering heparinized blood on Lymphoprep density gradients (Nycomed AS, Oslo, Norway) and centrifugation for 30 min at $600 \times g$ at room temperature. Cells in the interface were collected, washed twice in RPMI 1640, pH 7.4, and then resuspended in RPMI medium (pH 7.4) containing 3% AB^+ serum. Adherent monocytes were obtained by incubating the mononuclear cell fraction at 1×10^6 to 3×10^6 cells per coverslip for 30 min at 37°C in a 5% CO_2 atmosphere. The nonadherent cells were rinsed off by flushing the coverslip with incubating medium, and the adherent cells were kept in the medium and used in cytoadherence assays within 3 h after preparation. To check the purity of adherent monocytes, the adherent cells were scraped loose from a petri dish and stained with MAb to CD14 and FITC conjugate. Flow cytometric analysis showed that 76 to 86% of these cells were $CD14^+$.

Platelets were obtained by diluting platelet-rich plasma 1:1 (vol/vol) with RPMI medium (pH 7.4) and incubating 200 μl per coverslip at 37°C for 30 min. The coverslips were then rinsed with incubating medium.

All target cells were kept in incubating medium under culture conditions until used or fixed with 1% Formalin for 1 h, washed, and kept in PBS until used.

Cytoadherence assay. One hundred and fifty microliters of parasites having at least 10% trophozoite- or schizont-infected PRBC (T/S-PRBC), 3% Hct, in malaria culture medium was incubated on a coverslip of the target cells at 37°C for 1 h without agitation. The coverslips were rinsed with PBS, fixed briefly with 1% glutaraldehyde, washed, and stained with Giemsa for examination by light microscopy. The numbers of rosetting and nonrosetting PRBC bound per 100 target cells were calculated from the total number of PRBC among 500 target cells, both bound and not bound to PRBC.

To identify platelets, after cytoadherence was performed and the coverslip was fixed with glutaraldehyde and dried as above, the coverslip was then treated briefly with cold acetone, rinsed with PBS, stained with OKM5 MAb (2 μg/ml) and FITC conjugate, washed, mounted on a glass slide with 50% glycerol containing 10 μg of ethidium bromide per ml, and examined by UV microscopy.

On some occasions, the assay was performed with TM267R cultured at 1% Hct containing 2 to 3% T/S-PRBC. Some assays were also performed with rosetting-positive (R^+) and -negative (R^-) phenotypes of TM267R, which were obtained by enrichment as described previously (28) by gradient centrifugation on Histopaque (Sigma, St. Louis, Mo.) and 60% Percoll (Pharmacia, Uppsala, Sweden), respectively. The two parasite fractions were adjusted to 2 to 3% PRBC with uninfected RBC at 1% Hct before use.

Binding to CD36. Binding of PRBC to CD36 immobilized on a plastic surface was performed by coating a petri dish with 50 μl of soluble CD36, 2 μg/ml in PBS, for 1 h at room temperature. After being washed and incubated with 1% bovine serum albumin (BSA) for 30 min, 50 μl of parasite culture was added, incubated for 1 h at room temperature, washed, fixed, and stained as above. The number of PRBC bound to the CD36-coated surface was counted in an area of 10 mm^2, and the average number of PRBC per square millimeter was then calculated. Petri dishes coated with malaria culture medium containing 10% human serum or 1% BSA in PBS were included as controls.

Cytoadherence inhibition assay. Endothelial cells grown on coverslips for 18 to 20 h and adherent monocytes prepared for 1 h on coverslips as described above were incubated for 30 min at 37°C with MAb OKM5 to CD36 or MAb BMS101 or LB-2 to ICAM-1 at 4, 2, and 1 μg/ml or with combinations of both MAb (OKM5 and BMS101) at 2, 1, 0.5, and 0.25 μg of each MAb per ml. Target cells were also incubated with PBS, MAb to HLA-DR, and mouse IgG1 and IgG2 MAb as controls for binding. Coverslips were then rinsed with PBS, and cytoadherence was determined with the TM267R culture having 10% T/S-PRBC as described above. Five hundred target cells were examined, and the number of PRBC bound per 100 target cells was calculated and expressed as percent inhibition: % inhibition = $[(a - b)/a] \times 100$, where a is the number of PRBC counted in the presence of PBS and b is the number of PRBC counted in the presence of each MAb.

Target cell markers. One hundred microliters containing 3×10^5 to 5×10^5 endothelial cells, C32 or G361 melanoma cells, or adherent monocytes per ml was incubated with 2 μg of MAb OKM5 to CD36 or BMS101 to ICAM-1 per ml for 30 min at 4°C. These cells were washed twice in PBS and then incubated with $F(ab')_2$ fraction of rabbit anti-mouse immunoglobulin conjugated with FITC for 30 min at 4°C. After being washed twice in PBS, these cells were fixed for 5 min

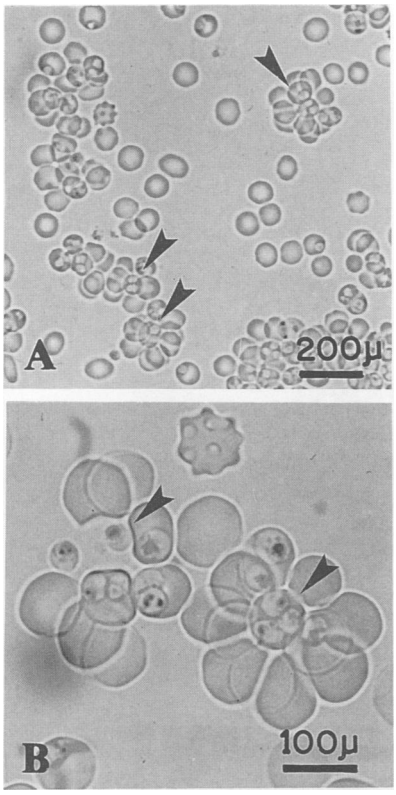


FIG. 1. Rosette formation by *P. falciparum*-infected RBC. Rosettes of *P. falciparum* isolate TM267R were examined by light microscopy with ×400 (A) and ×1,000 (B) objectives. Rosetting PRBC surrounded by several uninfected RBC were identified by the presence of malarial pigment (B, arrow). Bars: A, 200 μm; B, 100 μm.

in 500 μl of 0.5% paraformaldehyde in hemaline (Baker Instruments), diluted with an equal volume of PBS, and stored at 4°C. The number of target cells stained by each MAb was enumerated with a flow cytometer (FACSCAN; Becton Dickinson, Sunnyvale, Calif.) equipped with a 15-mW argon ion laser tuned to 488 nm. Five thousand cells were gated on forward light scatter for measurement of fluorescence intensity. Percent positive cells and fluorescence intensity were analyzed by integrated histogram (Consort 30 software; Becton Dickinson), with log amplification of fluorescence intensity for control and test sample distributions.

Differentiation of monocytes and cytoadherence. Adherent monocytes prepared on tissue culture dishes were kept under culture conditions for 0, 2, 4, and 24 h. The monocytes/macrophages were scraped loose from the culture dishes, fixed with 1% Formalin in PBS for 20 min, washed, and resuspended in 0.1% BSA in PBS. The cells were stained with MAb OKM5 to CD36 and BMS101 to ICAM-1 as described above and enumerated by flow cytometry. The monocytes/macrophages which had undergone differentiation during the incubation periods above were also used for binding with TM267R (10% T/S-PRBC) as described for the cytoadherence assay. Five hundred monocytes/macrophages, bound and not bound to PRBC, were examined, and the average number of PRBC bound per 100 monocytes/macrophages was calculated.

TABLE 1. Binding of *P. falciparum*-infected RBC and rosettes to different target cells[a]

Target cells	Isolate phenotype	Mean no. of PRBC/100 target cells ± SD		
		TM267R	TM178R	ND9
Endothelial cells				
Live	R^+	674 ± 307	667 ± 271	0
	R^-	1,693 ± 179	1,344 ± 98	151 ± 18
Fixed	R^+	3 ± 1	0	0
	R^-	135 ± 21	136 ± 11	0
Monocytes				
Live	R^+	25 ± 4	5 ± 2	0
	R^-	369 ± 151	428 ± 137	424 ± 163
Fixed	R^+	1 ± 1	0	0
	R^-	199 ± 44	126 ± 35	377 ± 47
C32 cells				
Live	R^+	0	0	0
	R^-	7 ± 2	3 ± 2	395 ± 21
Fixed	R^+	0	0	0
	R^-	5 ± 1	8 ± 1	268 ± 17
G361 cells				
Live	R^+	0	0	0
	R^-	0	0	198 ± 6
Fixed	R^+	0	0	0
	R^-	0	0	31 ± 4

[a] Assays were performed in duplicate, and three experiments were done on different occasions. Each value was calculated for 500 target cells examined, both bound and not bound to PRBC. Values are given separately for the R^+ and R^- phenotypes of each isolate.

RESULTS

***P. falciparum* isolates.** Two *P. falciparum* isolates, TM178R and TM267R, recently isolated in Thailand, naturally formed rosettes (Fig. 1) at various percentages. The mean percentage (range) of rosette formation by TM178R and TM267R in four observations was 6% (4 to 9%) and 51% (39 to 64%), respectively. These strains showed a wide range in intrinsic capacity to form rosettes, as suggested by the differences between the two isolates, which have been maintained in culture for 1 year (TM178R) or 5 months (TM267R). A nonrosette-forming isolate, ND9, maintained for 8 months in culture, was examined and included for comparison.

Cytoadherence characteristics of PRBC. Previous work on the cytoadherence to target cells of nonrosetting *P. falciparum*-infected RBC showed that cytoadherence was the result of interaction between parasite ligands and receptors on the target cells. We investigated whether *P. falciparum* from areas where it is endemic which formed rosettes during in vitro culture would also interact with other target cells despite the fact that the central PRBC was physically constrained by surrounding uninfected RBC. An in vitro cytoadherence assay with human umbilical vein endothelial cells as targets showed that these rosetting parasites were able to bind fresh but not Formalin-fixed endothelial cells (Table 1 and Fig. 2A). There was no difference between the two Thai isolates (TM267R and TM178R) of *P. falciparum* in rosette binding to endothelial cells despite the difference in rosette-forming capacity of the two isolates. However, compared

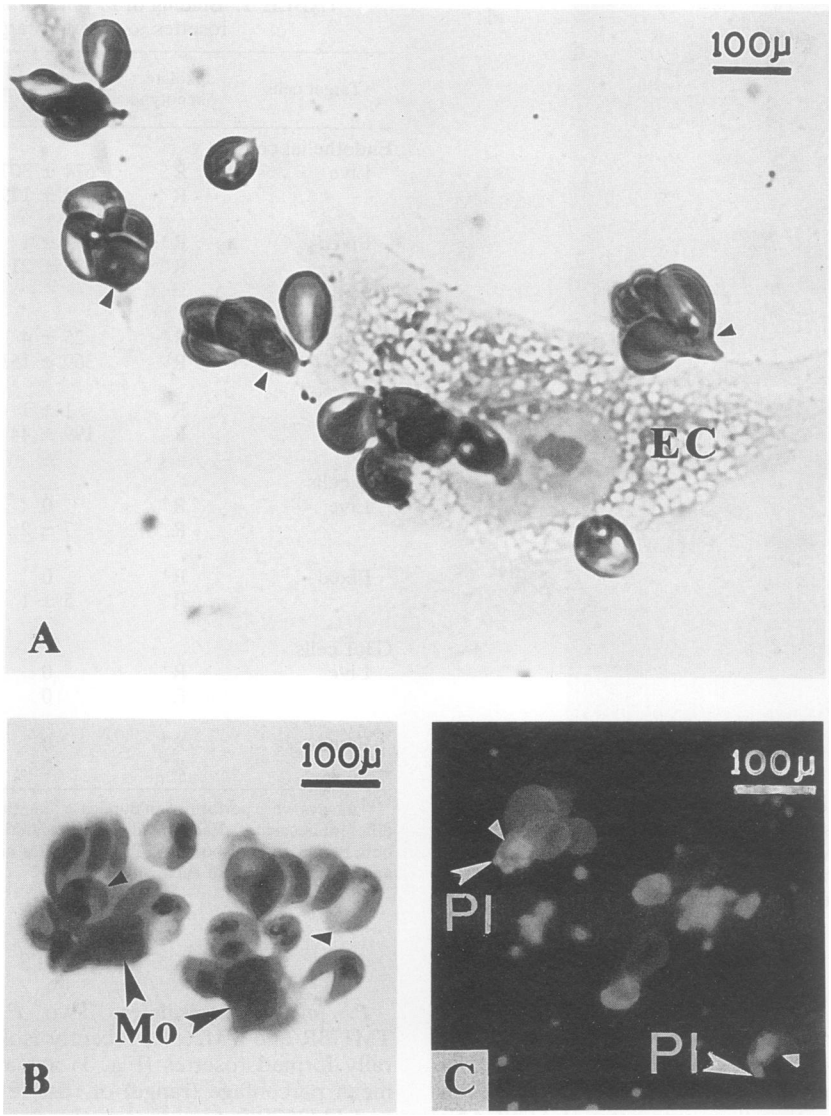


FIG. 2. Binding of rosette-forming PRBC to different target cells. The parasite used in cytoadherence assay was the TM267R isolate. Target cells were (A) endothelial cells (EC), (B) adherent monocytes (Mo; large arrowheads), and (C) platelets (Pl; large arrowheads). The rosette-forming PRBC bound to target cells are indicated by the small arrowheads (A and B) or counterstained with ethidium bromide (C).

with the above conditions, when the assay was performed with TM267R containing 2 to 3% T/S-PRBC at 1% Hct, the number of PRBC (as rosettes [R^+] or as nonrosettes [R^-]) bound to target cells was reduced; 81 R^+ and 119 R^- per 100 endothelial cells and 34 R^+ and 36 R^- per 100 adherent monocytes). When rosetting-positive and -negative phenotypes of TM267R were enriched in two separate fractions (85% R^+ and 98% R^-) and used for cytoadherence to endothelial cells and adherent monocytes at 2 to 3% T/S-PRBC with 1% Hct, a marked reduction in the number of PRBC bound to both target cells was observed (31 R^+ and 19 R^- per 100 endothelial cells; 5 R^+ and 25 R^- per 100 adherent monocytes). Further investigation showed that fresh but not Formalin-fixed monocytes and platelets obtained from peripheral blood bound rosettes from the two isolates of *P. falciparum* (Fig. 2B and C). Individual PRBC from these rosette-forming isolates reacted similarly with fresh and fixed endothelial cells and monocytes (Table 1). Similar results were obtained with the nonrosetting isolate ND9, although it bound to fixed monocytes but not endothelial cells.

Under various conditions, either high or low percent T/S-PRBC and Hct, we observed that neither of the two rosetting *P. falciparum* isolates showed binding to C32 melanoma cells or to G361 cells (Table 1). As shown in Table 1, the non-rosette-forming PRBC from ND9 bound to the C32 melanoma cells, the G361 cell line, and monocytes.

We also observed the ability of R^+ and R^- binding to CD36. The levels of R^+ and R^- binding were 24 and 79 per mm^2, respectively. PRBC did not bind to a plastic surface coated with malaria culture medium or 1% BSA in PBS.

Effect of MAb on cytoadherence. To test whether binding of rosetting *P. falciparum* to target cells involved the putative receptors CD36 and ICAM-1, we used MAb against the two proteins to block their receptors on endothelial cells and monocytes. With endothelial cells as targets, MAb OKM5 or

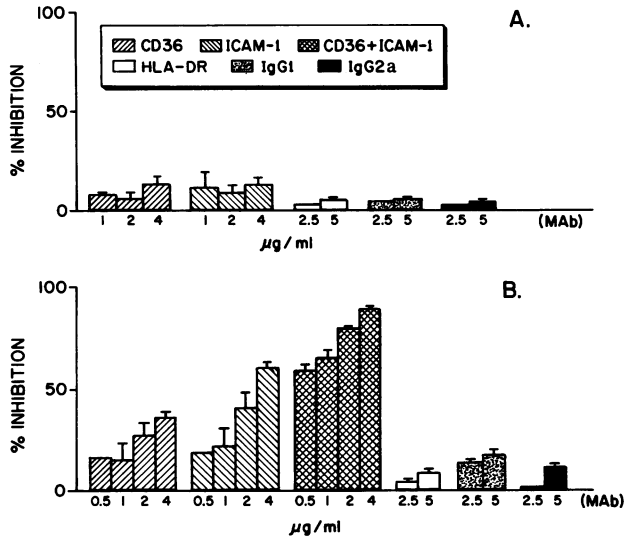


FIG. 3. Inhibition of cytoadherence by MAb to target cells. The parasite used in this assay was the TM267R isolate. Target cells were (A) endothelial cells and (B) adherent monocytes. MAb OKM5 to CD36 and MAb BMS101 to ICAM-1 were used at the indicated concentrations. Assays were done in duplicate, and each bar shows the mean percent inhibition ± standard deviation calculated from three experiments.

BMS101 showed no substantial blocking of PRBC binding (less than 20% inhibition at 4 μg of the MAb per ml) (Fig. 3A). Another MAb to ICAM-1 (LB-2) also did not block PRBC binding (data not shown). With monocytes as targets, both MAb OKM5 and BMS101 were observed to block the binding of PRBC in a dose-dependent fashion (Fig. 3B). MAb BMS101 had a stronger inhibitory effect on binding with monocytes (60% inhibition at 4 μg/ml) than did MAb OKM5 (35% inhibition at 4 μg/ml). The combination of MAb OKM5 and BMS101 increased inhibition of binding of PRBC to monocytes (Fig. 3B). Similarly, MAb LB-2 did not block PRBC binding to monocytes (data not shown). Neither MAb to CD36 nor to ICAM-1 was observed to substantially reverse the binding of PRBC to monocytes (10 to 20% reversal at 4 μg/ml; data not shown). Control MAb to HLA-DR, mouse IgG1, and mouse IgG2 had a weak or no inhibitory effect at similar concentrations.

Target cell markers and their relationship to the binding of PRBC. We examined the expression of CD36 and ICAM-1 on endothelial cells and monocytes as well as C32 and G361 melanoma cells by staining with MAb and using flow cytometry to determine the number of positive cells and intensity of staining. Endothelial cells appeared not to express CD36, as measured with MAb OKM5, but 68% of these cells expressed ICAM-1, as measured with MAb BMS101, with moderate to high levels of fluorescence intensity (Fig. 4A). Greater than 80% of the adherent monocytes expressed high levels of CD36 staining and moderate levels of ICAM-1 staining (Fig. 4B). C32 melanoma cells expressed ICAM-1 at very high levels, whereas 52% of these cells showed little expression of CD36, as measured by immunofluorescence (Fig. 4C). G361 cells did not express CD36, whereas 62% of these cells expressed ICAM-1 at low levels (Fig. 4D).

Monocyte differentiation and parasite cytoadherence. Using flow cytometry, we observed that freshly adherent monocytes expressed higher levels of CD36 than of ICAM-1, as measured with MAb OKM5 and BMS101, respectively (Fig.

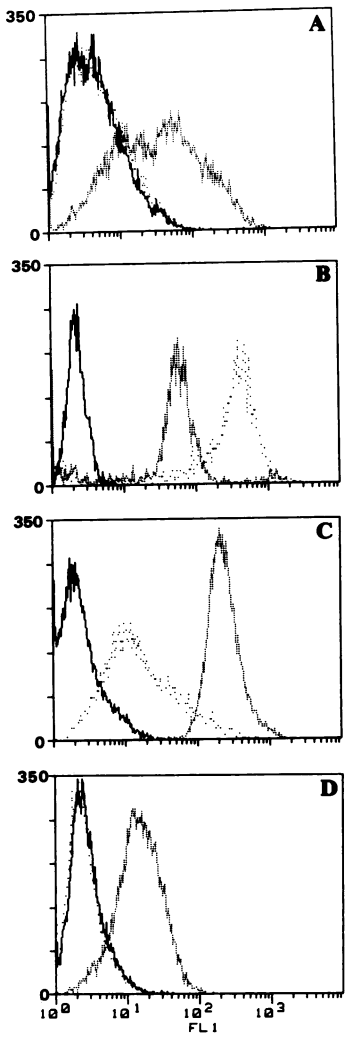
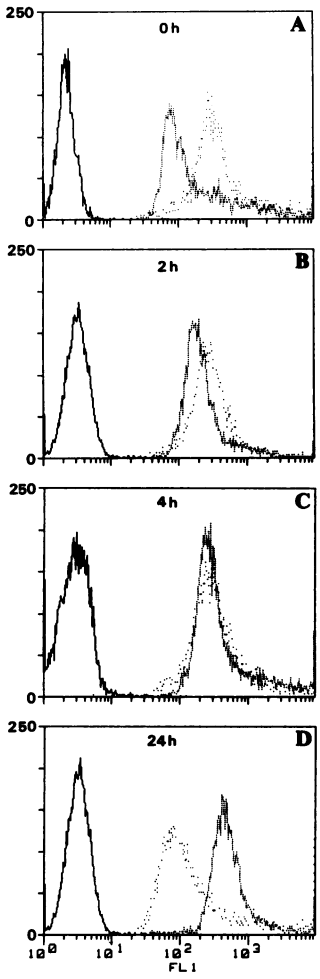


FIG. 4. Characteristics of target cell markers. Endothelial cells (A), adherent monocytes (B), C32 cells (C), and G361 cells (D) were studied for CD36 and ICAM-1 expression by immunofluorescence staining with MAb OKM5 and BMS101, specific to CD36 and ICAM-1, respectively, and examined by flow cytometry. ——, IgG MAb control; . . ., MAb to CD36; – – –, MAb to ICAM-1. FL1, fluorescence intensity; *y* axis, number of cells examined.

5A). At 2 h, the intensity of CD36 staining increased slightly (Fig. 5B) but then decreased thereafter, until by 4 h, it was comparable to the intensity of ICAM-1 staining (Fig. 5C). At 24 h after adherence, the differentiated monocytes/macrophages showed a marked decrease in CD36 intensity relative to ICAM-1, with an overall increase in ICAM-1 (Fig. 5D)—just the opposite of the relationship seen at 0 h. When cytoadherence of PRBC with these differentiating monocytes/macrophages was studied, we found that binding of the PRBC correlated well with the level of CD36 expression. By examining 500 monocytes/macrophages, both bound and not bound to PRBC, maximal binding of infected RBC was observed after 2 h of monocyte/macrophage differentiation, during which CD36 expression was maximal and the ICAM-1 (CD54) level remained low (Fig. 5B). Decreased binding of PRBC was observed after 4 h until at 24 h, when CD36 was at a minimum level but CD54 was at a maximum



FL1 CD36/ICAM-1	PRBC / 100 TARGETS
280/ 76	150 ± 10
301/202	212 ± 27
242/290	122 ± 39
82/417	109 ± 16

FIG. 5. Monocyte surface markers and cytoadherence during differentiation. Adherent monocytes were studied at 0, 2, 4, and 24 h for surface expression of CD36 (OKM5) and ICAM-1 (BMS101) by immunofluorescence staining and flow cytometry. Differentiating monocytes were also studied for cytoadherence with the TM267R isolate. The fluorescence intensity of CD36 and ICAM-1 and the number of PRBC per 100 monocytes at each period of differentiation are given in the right panel. PRBC values are means ± standard deviation calculated from two experiments, with each experiment done in duplicate. Symbols are the same as in Fig. 4.

level (Fig. 5D), the binding of PRBC was half that obtained at 2 h.

DISCUSSION

In this report, we have described the cytoadherence characteristics of rosette-forming *P. falciparum*-infected RBC with different target cells: endothelial cells, monocytes, platelets, and C32 and G361 melanoma cells. Although endothelial cells are the in vivo targets in the sequestration-related pathophysiological sequalae of falciparum malaria, monocytes have been used as an in vitro target cell model for cytoadherence (3, 15) because of their availability and well-characterized surface molecules. Two Thai *P. falciparum* isolates that formed rosettes at high and low frequencies were chosen for the cytoadherence studies. A nonrosetting Thai isolate was included for comparison.

In this study, we found that *P. falciparum*-infected RBC simultaneously formed rosettes and adhered to other target cells. Both rosettes and individual PRBC of the two rosetting *P. falciparum* isolates, TM267R and TM178R, adhered to live endothelial cells and monocytes. The number of PRBC binding in this case was not affected by differences in the percentage of rosette formation. However, the number of PRBC (R^+ and R^-) bound to target cells was affected by the percentage of T/S-PRBC used in the assay. A reduction in the number of both R^+ and R^- bound to target cells was observed when assays were performed with a low percent T/S-PRBC and separate fractions of R^+ and R^- phenotypes of TM267R. These results suggest that the parasite isolate TM267R used in this study is heterogeneous and contains different populations of parasites which have different characteristics for rosetting and binding to other target cells (R^+ C^+), that some parasites are rosetting but do not bind to other target cells (R^+ C^-), or that some are R^- C^+ and some are R^- C^-. Alternatively, the uninfected RBC in the rosettes interfere with cytoadherence under in vitro assay conditions when the shear force of blood flow does not exist.

Binding of PRBC to endothelial cells from passages 2 through 4 was consistent, as has been reported recently (25), but binding to fixed target cells compared with live cells was greatly reduced. The nonrosetting Thai *P. falciparum* isolate ND9 bound to endothelial cells and also adhered to fixed monocytes. Surprisingly, neither of the two rosetting *P. falciparum* isolates adhered to C32 or G361 melanoma cells, either as rosettes or as individual PRBC. Conversely, PRBC of the nonrosetting parasite ND9 did bind to the C32 and G361 cell lines. The G361 cell line has been reported previously not to bind PRBC (23). Our demonstration that ND9 bound to G361 cells therefore suggests a mechanism of cytoadherence of the ND9 isolate distinct from that reported previously (23).

Recent findings (27) indicate that the mechanism of binding of PRBC to form rosettes is different from that involved in cytoadherence to melanoma cells. Studies on the cytoadherence of *P. falciparum* have shown that there was no correlation between binding of PRBC with endothelial cells and C32 melanoma cells (4). These published data, together with our observation that the three Thai isolates bound differentially to different target cell types and also bound to purified CD36 immobilized on a plastic surface, suggest that the CD36 adherence ligand on PRBC can be independently expressed and is distinct from the ligand(s) for erythrocyte rosetting or endothelial cell or monocyte adherence.

Considerable research has been done by various laboratories to identify the cytoadherence receptors on selected target cells. Interpretation of previous work depends critically on the type of parasites and target cells, tissues, and experimental conditions used. Moreover, differences in cell surface marker expression on the same types of cells residing in different organs have been shown (11). At least three putative cytoadherence receptors (CD36, ICAM-1, and thrombospondin) have been reported on various target cell types. Varying degrees of importance have been accorded to these receptors—particularly CD36—in the interpretation of PRBC-target cell interaction.

We asked which of these putative cytoadherence receptors were present on endothelial cells and other cell types used in cytoadherence studies. We observed that CD36 was present on monocytes and C32 melanoma cells but was not detected on umbilical vein endothelial cells and G361 melanoma cells. ICAM-1 was detected at various levels on all four target cell types examined. Still, as we have reported previously, the rosetting *P. falciparum* isolates adhered to

endothelial cells and monocytes as both rosettes and individual PRBC but showed no binding to C32 and G361 melanoma cells.

To examine the relative importance of CD36 and ICAM-1 in cytoadherence by different target cells, we used specific MAb to these receptors in cytoadherence inhibition assays. There was no significant inhibition of binding of PRBC to endothelial cells by either of the two MAb, nor could any MAb reverse cytoadherence to the endothelial cells. Inhibition by the OKM5 MAb was not expected, since endothelial cells do not react with this MAb. Our finding is consistent with a previous observation (7) which suggests that other receptors may be important in the adherence of PRBC to the vascular endothelium. For monocytes, however, inhibition of PRBC binding was increased in a dose-dependent manner with MAb to CD36 or ICAM-1. The inhibitory effect of these MAb was additive when the two MAb were combined in the assay; however, complete inhibition was not achieved. With another MAb to ICAM-1 (LB-2; Becton Dickinson) at a range of concentrations similar to those of MAb BMS101, we found no inhibitory effect on either endothelial cells or monocytes. These results suggest that MAb to ICAM-1 obtained from different producing cell clones recognize different epitopes on the ICAM-1 molecule and hence have variable cytoadherence-inhibitory effects. The results also suggest that other receptors are involved in the adherence of PRBC to these target cells.

We interpret these observations with regard to the two rosetting *P. falciparum* isolates as follows. Although ICAM-1 was present on all four target cells, ICAM-1 was not by itself sufficient for binding of PRBC to endothelial cells. Furthermore, even though ICAM-1 was present on C32 and G361 cells, in our study with the rosette-forming *P. falciparum* TM267R and TM178R isolates, there was no binding of PRBC to these target cells. ICAM-1 did appear to contribute to cytoadherence with monocytes. CD36 appeared to be important on monocytes and possibly C32 melanoma cells. However, CD36, as defined by reactivity with the OKM5 MAb, appeared not to be involved in binding of PRBC to endothelial cells.

Binding of PRBC may vary with the functional status of receptors, depending on target cell type, state of differentiation, and physiological conditions. Our experiments with adherent monocytes/macrophages which showed up- and down-regulation of CD36 and ICAM-1 expression with increasing time after adherence indicated that the state of cell activation or differentiation may contribute to the relative availability of a specific receptor for binding of PRBC. These observations suggest the need for further investigation of the receptors and host factors involved in sequestration of *P. falciparum* and on the involvement of rosette formation in the pathogenesis of severe malaria.

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