Role of the *Plasmodium falciparum* Mature-Parasite–Infected Erythrocyte Surface Antigen (MESA/PfEMP-2) in Malarial Infection of Erythrocytes

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During intraerythrocytic growth of *Plasmodium falciparum*, several parasite proteins are transported from the parasite to the erythrocyte membrane, where they bind to membrane skeletal proteins. Mature-parasite-infected erythrocyte surface antigen (MESA) has previously been shown to associate with host erythrocyte membrane skeletal protein 4.1. Using a spontaneous mutant of *P. falciparum* that has lost the ability to synthesize MESA and 4.1-deficient erythrocytes, we examined growth of MESA(+) and MESA(−) parasites in normal and 4.1-deficient erythrocytes. Viability of MESA(+) parasites was reduced in 4.1-deficient erythrocytes as compared with that for normal erythrocytes, but MESA(−) parasites grew equally well in 4.1-deficient and normal erythrocytes. Cytoadherence of MESA(+) and MESA(−) parasitized normal and 4.1-deficient erythrocytes to C32 melanoma cells was similar, indicating that neither protein 4.1 nor MESA plays a major role in cytoadherence of infected erythrocytes. Localization of MESA in normal and 4.1-deficient erythrocytes was examined by confocal microscopy. MESA was diffusely distributed in the cytosol of 4.1-deficient erythrocytes but was membrane-associated in normal erythrocytes. These findings suggest that MESA binding to protein 4.1 plays a major role in intraerythrocytic parasite viability.

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and protein 4.1-deficient erythrocytes. We find that the expression of MESA in host erythrocytes lacking protein 4.1 results in accumulation of MESA in the erythrocyte cytosol, leading to poor survival of *P. falciparum*. Furthermore, the accumulation of MESA in the cytosol, but not at the membrane, of 4.1-deficient erythrocytes is consistent with the thesis that MESA is anchored to the erythrocyte membrane skeleton by binding to protein 4.1. Interestingly, the cytoadherence phenotype of MESA(+) and MESA(−)-infected normal and 4.1-deficient erythrocytes was similar. These findings enable us to show an important functional role for interaction between MESA and protein 4.1 for parasite viability but not for cytoadherence.

**MATERIALS AND METHODS**

**Parasites.** In vitro cultures of *P. falciparum* were maintained in A+ erythrocytes as previously described.20 IgG2Fg1 is a Brazilian isolate cloned at the National Institutes of Health (Bethesda, MD) and selected for increased cytoadherence.19 D6 is an isolate from Sierra Leone cloned by micromanipulation at the Walter Reed Army Institute of Research (Washington, DC).21 Two lines were established from this clone, D6-1 selected for knobs and increased cytoadherence and D6-2 maintained in culture for 6 months with no selections.19 D6-1 was recognized as MESA(−) and D6-2 as MESA(+).18 The MESA(+) line was cloned to yield the D63 line, and the MESA(−) line was cloned to yield the D6-1FS line, both by limited dilution at the Walter and Elisa Hall Institute of Medical Research (Melbourne, Australia). Analysis of the cloned D6-1FS line showed that the entire MESA gene had been deleted from the genome (Coppell et al, manuscript in preparation).

**Protein 4.1-deficient and normal erythrocytes.** The 4.1-deficient erythrocytes were obtained after informed consent from an individual who is homozygous for a structural rearrangement of the protein 4.1 gene, which results in deletion of the downstream AUG in 4.1 mRNA and complete deficiency of protein 4.1 in the RBCs.12 Normal erythrocytes were obtained from healthy volunteers. Both normal and 4.1-deficient erythrocytes were cryopreserved and stored in liquid nitrogen for subsequent use.22 The shape and mechanical properties of thawed cells, determined by both micropipette and ectactometry assays, were identical to those of fresh erythrocytes (D. Discher, personal communication, December 1994).

**Rabbit antisera.** Fragments of the MESA protein encompassing the GESKET repeat region at the amino terminus were cloned into the plasmid vector pTc (Invitrogen, San Diego, CA) to yield a fusion protein with a hexa-histidine amino terminal tag. Bacterial cultures were grown in overnight culture, induced with 1 mM IPTG, and cultured for 3 hours. After collection of the bacterial cells by centrifugation, the cells were lysed in guanidine hydrochloride and bound to nickel-chelate resin according to the manufacturer’s instructions (Qiagen, Chatsworth, CA). Purified fusion protein was eluted by pH change, and purity was determined to be greater than 99%. Rabbit antisera were injected with 50 to 100 μg/rabbit at monthly intervals for 3 injections, and blood was taken 14 days after the last boost. The serum was shown to contain antibodies that readily recognized the fusion protein and the MESA protein in immunoblots of parasite extracts.

**Immunofluorescent confocal microscopy.** Thin blood smears of mature trophozoite-infected erythrocytes were prepared from in vitro cultures, air-dried, and fixed with acetone/methanol. They were reacted at room temperature for 45 minutes with the anti-KAHRP monoclonal antibody (MoAb) 899 or anti-MESA diluted 1:20 or 1:100, respectively, in phosphate buffered saline (PBS), were washed 3 times in PBS, and were reacted for 45 minutes with fluorescein isothiocyanate-conjugated goat-antimouse or -antirabbit IgG (Molecular Probes, Eugene, OR) diluted 1:100 in PBS. Slides were again washed 3 times in PBS and mounted with SlowFade (Molecular Probes) and a coverslip for examination with a Zeiss Axioskop equipped with Nikon epifluorescence.

**Detergent extraction, immunoprecipitation, sodium dodecyl sulfate-gel electrophoresis, and autoradiography.** Detergent extraction, immunoprecipitation, sodium dodecyl sulfate-gel electrophoresis, and autoradiography were performed as previously described,23 using anti-MESA rabbit antibodies to immunoprecipitate 32P-labeled Triton X-100 insoluble extracts.

**Growth assays.** MESA(+) and MESA(−) parasites in normal erythrocytes were purified to greater than 80% either by Percoll gradient purification15 or by flotation in 0.5% gelatin/bicarbonate-free RPMI 1640 for 45 minutes.24 The fraction containing mature trophozoite-infected erythrocytes was washed once in bicarbonate-free RPMI and added to freshly thawed normal or 4.1-deficient erythrocytes to achieve comparable initial parasitemias on day 0. Starting parasitemias in normal and 4.1-deficient erythrocytes ranged from 1.4% to 5% and 1.5% to 4.6%, respectively, for MESA(+) and 2.3% to 7.4% and 1.2% to 7.8%, respectively, for MESA(−) parasites. Infected erythrocytes were cultured in vitro as above. This protocol establishes a synchronized culture system with a 48-hour cycle of erythrocyte rupture and parasite release, invasion of uninfected erythrocytes, and parasite maturation. Thin blood smears were made at the beginning of the assay and every 24 hours thereafter for the duration of the experiment (5 days). A total of 2,500 erythrocytes were counted per slide. The number of infected erythrocytes and the parasites' stage of development were recorded. Single assays were reproduced six times, and one duplicate assay and one triplicate assay were also performed. Significant statistical differences between means were determined by a two-way analysis of variance coupled to Fisher’s Least Significant Difference Test of Multiple Mean Comparisons. These analyses were performed using the Systat 5 computer program for the Macintosh (SYSTAT Inc, Evanston, IL).

**Gelatin enrichment assays.** Incubation of infected erythrocytes in 0.5% gelatin/bicarbonate-free RPMI 1640 is a technique that purifies trophozoite-infected cells with knobs from uninfected, ring-stage-infected, and knobless erythrocytes, based on the reduced deformability of knobbed infected erythrocytes.25 Assays were conducted in parallel with normal and 4.1-deficient erythrocytes infected with MESA(+) and MESA(−) parasites. Parasitemia in the enriched supernatant was determined by counting 2,500 Giemsa-stained erythrocytes by light microscopy.

**Cytoadherence assay.** Cytoadherence assays were performed as previously described19 but with the following modifications: infected normal or protein 4.1-deficient erythrocytes (parasitemia 2% to 6%) at 2% hematocrit (Hct) in bicarbonate-free RPMI 1640 were added to monolayers of unfixed C32 amelanotic melanoma cells (ATCC 1532; American Type Culture Collection, Rockville, MD) grown on Thermorax coverslips (Nunc, Naperville, IL) and were incubated for 1 hour at 37°C, with gentle rocking every 10 minutes. Coverslips were washed 3 times in bicarbonate-free RPMI 1640 and fixed in 2% glutaraldehyde/PBS before Giemsa staining. Results were recorded as the number of infected erythrocytes bound per 100 melanoma cells.

**RESULTS**

**Invasion, development, and differentiation of *P. falciparum*.** Data on invasion from a large series of experiments using MESA(+) and MESA(−) parasites and normal and protein 4.1-deficient cells are shown in Fig 1. There was no statistically significant difference in the invasion and devel-
Invasion, development, and multiplication of MESA(+) and MESA(-) *P. falciparum* parasites in normal and 4.1-deficient erythrocytes. The same data is presented in different forms in (A) and (B) and in (C) and (D) for comparative purposes. Normal erythrocytes infected with mature trophozoite-stage parasites were purified to greater than 80% and added to either normal or 4.1-deficient erythrocytes (day 0). A total of 2,500 erythrocytes were counted from Giemsa-stained thin blood smears every 24 hours to determine parasitemia and stage of parasite development. Day 1 represents initial invasion, day 2 represents maturation to trophozoites, day 3 represents reinvasion, and day 4 represents maturation to trophozoites. Bars represent standard error of the mean. Significant statistical differences between means were determined by a two-way analysis of variance coupled to Fisher's Least Significant Difference Test of Multiple Mean Comparisons. Starting parasitemias in normal and 4.1-deficient erythrocytes ranged from 1.4% to 5% and 1.5% to 4.6%, respectively, for MESA(+) and from 2.3% to 7.4% and 1.2% to 7.8%, respectively, for MESA(-) parasites. The value at day 0 is a mean value representing a normalization of the initial parasitemia of 8 replicates. Values for subsequent days are normalized to day 0 and represent a percentage of the initial parasitemia. Each point represents 5 to 8 replicates. Significant differences between groups on a given day are indicated by an asterisk (*, P < .01 to .05). (A), growth of MESA(+) (x) and MESA(-) (△) parasites in normal erythrocytes; (B), growth of MESA(+) (x) and MESA(-) (△) parasites in 4.1-deficient erythrocytes; (C), growth of MESA(+) parasites in normal (■) and 4.1-deficient (○) erythrocytes; (D), growth of MESA(-) parasites in normal (■) and 4.1-deficient (○) erythrocytes.

opment of MESA(+) and MESA(-) parasites in normal erythrocytes on days 1 to 3 (Fig 1A). In marked contrast, rates of invasion and development in protein 4.1-deficient erythrocytes for MESA(+) parasites were significantly lower than those for MESA(-) parasites on days 1 to 3 (Fig 1B).

Direct comparison of the growth of MESA(+) parasites in normal and protein 4.1-deficient erythrocytes (Fig 1C) shows that, on day 1, the parasitemia of normal erythrocytes reached approximately 180% of the starting value; whereas, in the first round of invasion of 4.1-deficient erythrocytes, the parasitemia decreased to approximately 86% of initial parasitemia. In contrast, the parasitemia of MESA(-) parasites in normal erythrocytes reached 150% of the starting parasitemia, and in protein 4.1-deficient erythrocytes reached approximately 200% of the starting parasitemia (Fig 1D). Thus, although there was no statistical difference on day 1 between normal and 4.1-deficient erythrocyte invasion by MESA(-) parasites (1.5-fold and two-fold increase in parasitemia, respectively; see Fig 1D), there was a statistically significant difference between normal and protein 4.1-deficient erythrocyte invasion by MESA(+) parasites (1.8-fold increase in parasitemia versus a 0.85-fold decrease, respectively; see Fig 1C).
The second round of invasion (day 3) resulted in a further dramatic decrease in the ability of MESA(+) parasites that matured in 4.1-deficient RBCs to subsequently invade other 4.1-deficient RBCs. There was a reduction from 72% of starting parasitemia to 32% in the infection of these erythrocytes. At the end of 4 days (2 multiplicative cycles), infection of 4.1-deficient erythrocytes by MESA(+) parasites was 14% of the starting parasitemia, whereas infection by MESA(−) parasites was 97% of the starting parasitemia. In comparison, at the end of 4 days, infection of normal RBCs was 127% of the starting parasitemia for MESA(−) and 265% for MESA(+).

Development of the intraerythrocytic MESA(+) parasites from ring stage to trophozoite stage was also disrupted in the 4.1-deficient RBCs. There was an approximately 15% decrease in parasitemia between the ring stage and trophozoite stage (days 1 to 2), indicating that many parasites were unable to undergo normal development. In contrast, the MESA(−) parasites matured normally in 4.1-deficient erythrocytes with no decrease in parasitemia.

In spite of the observed decreases in parasitemia, moribund parasites that had invaded 4.1-deficient erythrocytes but had not developed further were observed in fewer than 1% of the MESA(+) -infected erythrocytes. Degenerated MESA(+) trophozoites that were free from 4.1-deficient erythrocytes were observed at the rate of approximately 1/100 erythrocytes, whereas, with MESA(−) parasites and with normal erythrocytes, these were not observed. However, surviving MESA(+) parasites in 4.1-deficient erythrocytes showed no morphological abnormalities at any developmental stage at the light microscopy level of resolution. Typical ring morphology, multiply invaded cells, and parasite "applique" forms were noted in both normal and protein 4.1-deficient erythrocyte populations. Differentiation to the multinucleated schizont, and the number of merozoites formed did not appear to differ between normal and 4.1-deficient host erythrocytes.

Cytoadherence. There were no differences between the binding to C32 melanoma cells by normal and 4.1-deficient erythrocytes infected with either MESA(+) or MESA(−) clones. The in vitro cytoadherence phenotype of normal and 4.1-deficient infected erythrocytes did not differ when a MESA(+) ItG2F6 parasite clone selected for increased cytoadherence was assayed for binding to C32 melanoma cells (Fig 2). In six replicates, the mean number of ItG2F6-infected erythrocytes binding per 100 melanoma cells was 236 for normal erythrocytes and 240 for 4.1-deficient erythrocytes. Both MESA(+) and MESA(−) lines derived from the D6 clone had lower cytoadherence to melanoma cells than the ItG clone, but their cytoadherence phenotypes were similar to each other. In a triplicate experiment, the cytoadherence phenotype was qualitatively similar for either normal or 4.1-deficient erythrocytes infected with MESA(+) D6-3 (X = 23 and X = 17, respectively) and D6-IFS MESA(−) parasites (X = 43 and X = 49, respectively; data not shown).

Parasite antigen localization. Fluorescent confocal laser scanning microscopy was used to localize MESA and KAHRP in normal and 4.1-deficient erythrocytes infected with trophozoite stage parasites. There was no reactivity of uninfected erythrocytes in these preparations with either the anti-MESA or anti-KAHRP reagents. MESA was associated with the membrane of normal infected erythrocytes, as previously described, but was diffusely distributed in the cytosol of 4.1-deficient infected erythrocytes (Fig 3).

The localization of KAHRP was apparently the same in normal and 4.1-deficient erythrocytes. The reactivity of MoAb 89 was localized at the erythrocyte membrane in a punctate pattern in normal erythrocytes, as previously described, and also in the 4.1-deficient erythrocytes (Fig 4). Further evidence of the knobby phenotype of both normal and protein 4.1-deficient infected erythrocytes was provided by gelatin enrichment assays. This separation, which depends on the knob phenotype of the infected erythrocyte, showed that both MESA(+) and MESA(−) trophozoite stage parasites in normal and 4.1-deficient erythrocytes remained suspended in the supernatant, indicating that knobs were present in all these infected cell populations (data not shown).

Immunoprecipitation analysis. Previous work has shown an association between MESA and protein 4.1. To confirm this, we performed experiments in which synchronized cultures of infected erythrocytes were biosynthetically labeled with 32P04 and extracted with Triton X-100. Triton X-100-insoluble extracts were immunoprecipitated with rabbit serum raised against the amino terminal repeat region of MESA. Clear evidence of coprecipitation of MESA with the 80-kD protein 4.1 was obtained (Fig 5).

DISCUSSION

The function of MESA in the life cycle of intraerythrocytic P. falciparum parasites and the consequences of its association with erythrocyte skeletal protein 4.1 have yet to be delineated. Previous studies of P. falciparum infection of 4.1-deficient erythrocytes have shown reduced viability of parasites in erythrocytes with this skeletal abnormality, either through reduced invasion or reduced growth and multiplication. However, neither of these studies examined the role of any specific parasite-encoded proteins in this phenomenon.

Our studies indicate that the expression of MESA adversely affects the growth of parasites in 4.1-deficient erythrocytes. There were statistically significant differences between normal and 4.1-deficient erythrocytes in the growth of MESA(+) parasites but not with MESA(−) parasites. Both invasion by MESA(+) parasites of protein 4.1-deficient erythrocytes and intraerythrocytic maturation to the trophozoite stage were reduced.

Morphology of intraerythrocytic parasites that survived from the ring stage to schizogony (formation of the multinucleated schizont before rupture of the erythrocyte) appeared typical in either normal or 4.1-deficient erythrocytes. Several gametocytes (the stage that is infective for mosquitoes) were observed, suggesting that the transmissibility of parasites in 4.1-deficient erythrocytes is not lost. The number of nuclei in schizonts of both MESA(+) and MESA(−) lines ranged from 8 to 18 in 4.1-deficient erythrocytes and from 8 to 24 in normal erythrocytes.
Fig 2. In vitro cytoadherence of normal (A) and protein 4.1-deficient (B) infected erythrocytes to C32 melanoma cells. Note the elliptocytic morphology of the cytoadherent protein 4.1-deficient infected erythrocytes as compared with that of the normal infected erythrocytes. Infected normal or protein 4.1-deficient erythrocytes (parasitemia, 2% to 6%) at 2% Hct in bicarbonate-free RPMI 1640 were added to monolayers of unfixed C32 amelanotic melanoma cells (ATCC 1532) grown on Thermapex coverslips and were incubated for 1 hour at 37°C with gentle rocking every 10 minutes. Coverslips were washed 3 times in bicarbonate-free RPMI 1640 and fixed in 2% glutaraldehyde/PBS before Giemsa staining.
In spite of seemingly normal schizogony in 4.1-deficient erythrocytes, reinvasion by MESA(+) parasites of 4.1-deficient erythrocytes on day 3 was severely reduced. A number of mechanisms may account for this phenomenon. The accumulation of MESA in the host erythrocyte cytosol may affect the viability of merozoites released from these cells or in some way impair their ability to invade, or the accumulation of MESA may cause the membrane of 4.1-deficient erythrocytes to rupture prematurely, leading to release of merozoites that are not fully functional.

The development and differentiation of those MESA(+) parasites that survived in 4.1-deficient erythrocytes appeared normal at the light microscopy level of resolution, but we know that MESA accumulates in the cytosol of 4.1-deficient erythrocytes and that parasites that mature in such an environment do not as efficiently maintain the cycle of erythrocytic infection. The observed effect may be related to toxicity of unbound MESA. Alternatively, the accumulation of unbound protein may affect vital parasite interactions with the host erythrocyte. Protein transport through the erythrocyte cytosol may be impeded, or accumulated MESA protein in the erythrocyte cytoplasm may interfere with elaboration of the new parasitophorous vacuolar membrane or with the parasite’s metabolism of hemoglobin.

Our results may serve to clarify the discrepancies between the reports of Chishti et al\(^4\) and Schulman et al\(^4\) regarding parasite invasion of protein 4.1-deficient erythrocytes. Chishti et al\(^3\) reported reduced invasion of 4.1-deficient erythrocytes, whereas Schulman et al\(^4\) reported normal rates of invasion on day 1 but subsequent disrupted growth rates. We can only speculate on the MESA phenotype of parasites used in these two studies, and it is possible there are differences in the extent of MESA deletions between laboratory strains. We observed reduced invasion of 4.1-deficient erythrocytes by MESA(+) parasites, which became more pronounced in the second round of invasion, but invasion of 4.1-deficient erythrocytes by MESA(−) parasites did not differ significantly from invasion of normal erythrocytes. Additionally, there is not consistency between our results and those of Schulman et al\(^4\) with respect to MESA and parasite survival throughout the erythrocytic cycle. We observed either normal invasion and growth or reduced invasion and growth in 4.1-deficient erythrocytes depending on the MESA phenotype. Schulman et al\(^4\) reported normal invasion (as determined by \(^3\)H-hypoxanthine incorporation) but reduced growth (as determined by visual counting) in 4.1-deficient erythrocytes using parasites of indeterminant MESA phenotype.

Schulman et al\(^4\) suggested two mechanisms by which erythrocyte skeletal protein defects could disrupt the maturation of \textit{P. falciparum}. First, the parasite might require normal erythrocyte skeletal proteins for the construction of its own membranes. Alternatively, abnormal host erythrocyte skeletal proteins could fail as receptors or targets for parasite-encoded proteins that then interfere with the development of the parasite. Our results suggest that the second alternative, in particular, may be operative.

The results reported here are consistent with the proposition that the accumulation of unbound MESA in the erythrocyte cytosol is detrimental to the parasite. The evidence for this is that maintenance of the intraerythrocytic cycle of MESA(−) parasites in 4.1-deficient erythrocytes is not significantly different from that in normal erythrocytes, whereas the ability of MESA(+) parasites to continue the intraerythrocytic cycle in protein 4.1-deficient RBCs is reduced. In addition, however, we observed an initial reduction in the ability of MESA(+) parasites and not MESA(−) parasites, purified in normal erythrocytes, to invade 4.1-deficient RBCs. What role, if any, MESA might have in the invasion of erythrocytes has yet to be identified. MESA has not been
detected in merozoites, nor has a role for MESA in invasion been previously reported. We note that the chromosomal deletion which resulted in the loss of MESA expression is more extensive than merely the loss of the MESA gene, and this may have led to the loss of other as yet unrecognized proteins (Bennett and Coppel, manuscript in preparation). Thus, we cannot exclude the possibility that an unrecognized parasite protein may be responsible for the differences we observed in invasion of 4.1-deficient RBCs by MESA(+) and MESA(-) parasites. However, it is highly likely that the differences observed in MESA(+) versus MESA(-) parasite survival in protein 4.1-deficient erythrocytes are because of MESA, since there is unequivocal evidence for its interaction with protein 4.1.

Biochemical studies support our observations, made by immunofluorescence, of the interaction between MESA and protein 4.1. Lustigman et al showed coprecipitation of MESA and protein 4.1 with both anti-MESA and polyspecific anti-erythrocyte antibodies. We have confirmed their results by immunoprecipitation with anti-MESA antiserum, which coprecipitates 32P-labeled MESA and protein 4.1.

The absence of protein 4.1 from the RBC does not appear to affect either the formation or distribution of knobs. Ultrastructural and biochemical data suggest that KAHRP and the "electron dense cups" that compose knobs are anchored to the erythrocyte cytoskeleton. Leech et al showed that the insoluble residues of infected erythrocyte membranes extracted in 2% Triton X-100 in 0.6 mmol/L KCl contained "electron dense cups" and KAHRP, as well as host erythrocyte proteins spectrin, actin, and protein 4.1. Chishti et al identified spectrin, dematin, and protein 4.1 in highly enriched preparations of cytoskeleton-associated knobs. Direct binding studies using recombinant fragments of KAHRP suggested that a specific region of KAHRP associates with spectrin. We did not specifically examine the association of protein 4.1 with KAHRP, but our findings with protein 4.1-deficient cells suggest that such binding is not quantitatively important.

In vitro cytoadherence is a model for the phenomenon of in vivo sequestration, by which trophozoite-infected erythrocytes adhere to postcapillary venular endothelium, a prelude to the syndrome of cerebral malaria, the most serious complication of falciparum malaria. Our data show that MESA and protein 4.1, either alone or in association with each other, have no effect on the level of cytoadherence measured in static assays of infected erythrocytes to C32 melanoma cells. The particular cytoadherent interaction we have assayed in these studies is presumably between the parasite ligand
PIEMP1 (P. falciparum erythrocyte membrane protein 1) and the cellular receptor CD36, 26, 30 We have no data on whether MESA or protein 4.1 may be involved in other interactions between parasitized cells and cytoadherence receptors such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM), endothelial-leukocyte adhesion molecule (ELAM), or thrombospondin. PIEMP1 shares some biochemical and immunochemical properties with MESA, 26 but its association with specific erythrocyte skeletal protein(s) has yet to be determined. Our results indicate that, in contrast with MESA, which is associated with the host erythrocyte membrane solely by its interaction with protein 4.1, PIEMP1 or another adhesion ligand on the surface of infected erythrocytes is independent of protein 4.1.

The results reported here further illustrate the complexity of the intraerythrocytic malaria parasite’s interaction with the host erythrocyte membrane. An abnormal erythrocyte membrane has specific consequences for the normal targeting of a particular parasite-encoded protein and may not affect other parasite-induced modifications of the RBC. Our results suggest that the production of one parasite protein, MESA, is deleterious to parasite survival in 4.1-deficient erythrocytes. Further investigations of the function of MESA and the consequences of its association with protein 4.1 will contribute to our understanding of the mechanism by which MESA accumulation, in the absence of protein 4.1, is detrimental to intraerythrocytic P. falciparum parasites. It may be that interference with the interaction between parasite-encoded proteins and the RBC membrane skeleton could provide a previously unsuspected means of limiting malarial infection.

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