In Vivo Removal of Malaria Parasites From Red Blood Cells Without Their Destruction in Acute Falciparum Malaria

By Brian J. Angus, Kesinee Chotivanich, Rachenee Udomsangpetch, and Nicholas J. White

During acute falciparum malaria infection, red blood cells (RBC) containing abundant ring-infected erythrocyte surface antigen (Pf 155 or RESA), but no intracellular parasites, are present in the circulation. These RESA-positive parasite negative RBC are not seen in parasite cultures in vitro. This indicates that in acute falciparum malaria there is active removal of intraerythrocytic parasites by a host mechanism in vivo (probably the spleen) without destruction of the parasitized RBC. This may explain the observed disparity between the drop in hematocrit and decrease in parasite count in some hyperparasitemic patients. The fate of these “once-parasitized” RBC in vivo is not known.

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RESULTS

*Laboratory isolate.* Glutaraldehyde-fixed or methanol-fixed thin blood films of ring-infected RBCs from a laboratory parasite strain TM267R were tested with pooled sera of *P. falciparum* immune donors, and the human MoAb 33G2 to RESA. Both antibodies reacted with only the membrane of ring-infected RBCs and gave similar intensity and pattern of staining. This indicated that fixation of blood films with either glutaraldehyde or methanol provided equal antibody access to the RESA and that immune sera could substitute for the MoAb in routine staining.

All sera preabsorbed with AB+ RBCs showed no reactivity with normal RBCs in the indirect immunofluorescence assay. Serum from a *P. vivax*-infected patient and a healthy donor did not stain the ring form-infected RBCs. None of the antibodies stained uninfected RBCs in the TM267R parasite culture.

*Acute malaria.* Of the 48 patients studied, 26 had severe falciparum malaria (12 cerebral malaria, 3 acute renal failure, 1 severe anemia, 2 jaundice, 8 hyperparasitemia) and the other 22 had uncomplicated malaria. Four patients with severe malaria (15%) died. The geometric mean (range) parasite counts were higher in the severe malaria group: 174,830/μL (3,010 to 1,210,920) compared with the uncomplicated group; 26,540/μL (2,070 to 221,560); *P < .05*. The mean (standard deviation [SD]) hematocrit was similar in the severe and uncomplicated malaria groups: 32 (9)% and 35 (8)%, respectively. The clinical and laboratory values for the patients with severe malaria are shown in Table 1. Thin blood films from malaria patients on admission that contained bright ethidium bromide-stained parasite nucleic acid were then stained with the pooled immune sera or MoAb (g/dL) (5.5-15.2) that contained bright ethidium bromide-stained parasite nucleic acid were then stained with the pooled immune sera or MoAb (g/dL) (5.5-15.2). Five patients had severe disease and three had severe disease (*P = .011*). There was no significant difference in the number of RESA-RBC or measures of diseases severity between the antibody positive and antibody negative patients, although there was a significantly lower parasitemia in the antibody positive patients (*P = .009*).

<table>
<thead>
<tr>
<th>Table 1. Summary of Clinical and Laboratory Variables in 26 Patients With Severe Falciparum Malaria (median, range unless indicated)</th>
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<tbody>
<tr>
<td>Temperature (°C)</td>
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<td>Pulse (beats/min)</td>
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<td>Systolic blood pressure (mm Hg)</td>
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<td>Respiratory rate (breaths/min)</td>
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<td>Glasgow coma score</td>
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<tr>
<td>Parasite count/mL</td>
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<tr>
<td>RESA-RBC count/μL</td>
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<td>Ratio parasite count to RESA-RBC geometric mean (range)</td>
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<td>Serum creatinine (mg/dL)</td>
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<td>Total bilirubin (mg/dL)</td>
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<td>SGOT (U/L)</td>
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<td>SGPT (U/L)</td>
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<td>White cell count (cells × 10³/L)</td>
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<td>Hemoglobin (g/dL)</td>
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<td>Platelet count (cells × 10⁹/L)</td>
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**DISCUSSION**

The objective of these studies was to seek evidence of previous parasitization in unparasitized RBCs in acute falciparum malaria. The parasite-derived antigen Pf 155 or RESA was chosen because it is associated with dense granules in the apical part of the merozoite and is deposited in the erythrocyte membrane during invasion. RESA is, therefore, present in the RBC membrane from a very early stage of infection and acts as a “footprint” of RBC parasitization by a *P. falciparum* parasite. RESA is a well-characterized polypeptide antigen that is highly conserved and is immunogenic in man. Immune sera and MoAb 33G2 gave a distinct-
REMOVAL OF MALARIA PARASITES FROM RED CELLS

Fig 1. Immunofluorescence photomicrograph (original magnification × 1,000) stained with ethidium bromide and FITC-conjugated anti-RESA demonstrating a parasitized RBC on the left and a RESA positive, parasite negative RBC on the right.

Fig 2. Linear regression analysis of parasitemia with the number of RESA positive, parasite negative RBCs (RESA-RBC) (mean, 95% confidence interval for the slope).

tive pattern of rim fluorescence over the glutaraldehyde-fixed erythrocyte membrane, but not on unfixed cells. RESA is first synthesized within mature trophozoites and accumulates within merozoites. When RBCs from acute malaria patients were stained with pooled immune serum or MoAb3362, the pattern of antibody binding on some of the unparasitized RBCs was typical of RESA staining. Pooled immune serum is likely to have recognized a number of different parasite antigens, but the relative immunodominance of RESA was such that the two patterns of staining appeared identical.

There are three possible explanations for these observations. First, that in some cases, the merozoites invade the RBCs, then die spontaneously and are degraded rapidly within the cytoplasm. Second, that the young parasites are extruded actively by the RBC (either alive or dead), and third, that the parasites are somehow extracted from the RBC leaving it intact. If the parasites had simply died, then some DNA degradation products would still be expected to have stained by the ethidium bromide. This was not observed. The discrepancy between the numbers of RESA positive, parasite negative cells seen in vivo and in vitro suggests that active extrusion of parasites by the RBCs is unlikely to be quantitatively important. The most likely explanation is that host defense responses (probably the spleen) are involved in removal of intraerythrocytic parasites in vivo. The spleen normally removes residual host nuclear material from erythrocytes, but how it recognizes damaged intraerythrocytic parasites is not known.

The possibility that immature or killed intraerythrocytic
parasites could be removed from within RBCs either by phagocytic cells or by active extrusion without their destruction, was first raised by observations in experimental simian malaria by Conrad and Dennis. 

This was further supported by ultrastructural studies of the spleen in Rhesus monkeys infected with \textit{P. knowlesi}. 

Human neutrophils in vitro have also been shown to be capable of extracting \textit{P. falciparum} parasites from RBCs, leaving the RBCs intact and parasitized RBCs treated with antimalarial drugs have been recorded extruding dead trophozoites. 

This evidence in experimental systems supports our findings that removal of intraerythrocytic parasites occurs naturally in vivo when the host immune system can act, but not under in vitro parasite culture conditions in the absence of leukocytes and the spleen. It is likely that the parasites removed were in the first 24 hours of their 48-hour asexual life cycle because the morphology of the RESA positive cells was normal. In the second half of the asexual life cycle, cytoadherence takes place, and these adherent RBCs would have remained sequestered in the microcirculation whether or not they were still parasitized. 

As most of the intraerythrocytic hemoglobin is consumed by mature parasites, the hemoglobin concentration of the once parasitized cells was probably not reduced markedly. Thus, in the short-term, these once parasitized and antigenically marked RBCs, which occur in numbers approximately similar to the parasitized RBCs, will contribute to oxygen carriage and delivery, but their ultimate fate is not known. RESA is not expressed on the cell surface, 

so increased antibody binding and thus immune recognition would not be expected, but it is associated with the RBC cytoskeleton (through binding with spectrin) and could theoretically alter RBC deformability leading to splenic removal. Further study of the survival and the effects of different antimalarial drugs on the numbers of these cells in the circulation will be required.

**ACKNOWLEDGMENT**

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