Decreased Plasma Gelsolin Levels in Patients With Plasmodium falciparum Malaria: A Consequence of Hemolysis?

By David B. Smith, Paul A. Janmey, James A. Sherwood, Russell J. Howard, and Stuart E. Lind

Mammalian plasma contains a high-affinity actin-binding protein, plasma gelsolin, that severs actin filaments. Destruction of erythrocytes could result in the release of erythrocyte cytoskeletal actin into the plasma where it could bind to gelsolin. If the clearance of actin-gelsolin complexes exceeds its synthesis, lowering of the plasma gelsolin concentration might follow. To test this hypothesis, we measured plasma gelsolin levels in patients with falciparum malaria, a disease where at least part of the hemolysis takes place in the intravascular space and that is usually not accompanied by dysfunction of other organs. Two functional gelsolin assays showed that the mean plasma gelsolin concentration of 18 Nigerian children with Plasmodium falciparum malaria was <50% (P < .001) of healthy Nigerian control subjects tested at the same time. Patients with pneumonia and febrile seizures also had depressed gelsolin levels, which indicates that factors other than hemolysis can lower gelsolin concentrations. Gelsolin levels were measured in 11 patients from The Gambia with P falciparum malaria before and approximately 3 weeks after treatment. In all cases the gelsolin level increased after treatment. To confirm the hypothesis that hemolysis can result in a lowering of plasma gelsolin levels, hemolysis was induced in rabbits, either acutely (by the injection of human serum) or subacutely (by the administration of phenylhydrazine). A fall in plasma gelsolin levels was seen, the rate of fall differing with the extent of hemolysis. Affinity adsorption of plasma from animals undergoing acute hemolysis with Sepharose beads coupled to the actin-binding protein DNase I, followed by immunoblotting of adherent proteins with antiactin antiserum demonstrated the presence of actin in circulating rabbit plasma. These studies suggest that under some conditions components of the red cell cytoskeleton are exposed to plasma proteins and that accelerated clearance of actin-gelsolin complexes may explain in part the depressed plasma gelsolin levels seen in patients with falciparum malaria.

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The anatomy of the RBC has been carefully studied morphologically and biochemically. The mature erythrocyte may be thought of in terms of three anatomic domains: the cytosol, the membrane skeleton, and the plasma membrane. Hemolysis may expose these structures to plasma where each may interact in a specific manner with different plasma proteins. For example, the cytosolic protein hemoglobin is well known to bind to haptoglobin, and free hemin to hemopexin and albumin. Phospholipids of the inner surface of the erythrocyte membrane, particularly phosphatidylycerine, may interact with plasma clotting factors to initiate or promote thrombosis. To date there has been little study of the interactions of the red cell's cytoskeletal proteins with plasma proteins. Like all mammalian cells, erythrocytes contain actin. Unlike other cells in which approximately 50% of the cellular actin is polymerized into filaments and 50% is unpolymerized, erythrocyte actin is virtually all polymerized, and there are relatively few monomers.

Mammalian plasma contains an extracellular form of the actin filament—severing protein gelsolin. The high affinity of plasma gelsolin for F-actin (K > 109/mol/L) suggests that its physiological role is likely related to its actin-binding properties. If so, plasma gelsolin would serve to shorten actin filaments released from dying cells and/or to clear actin from the circulation, as haptoglobin does hemoglobin.

Because gelsolin-actin complexes are cleared more rapidly from the rabbit circulation than is free gelsolin, release of actin from large numbers of dying cells might lead to a decline in the plasma gelsolin concentration. We therefore measured gelsolin levels in patients with falciparum malaria. Because some of the hemolysis of this disorder is thought to take place in the intravascular space, it seemed likely that erythrocyte cytoskeletal proteins would be exposed to plasma proteins. Unlike other forms of human malaria, the liver is not infected in Plasmodium falciparum malaria, and relatively little damage to other organs is encountered. Depressed levels of plasma gelsolin can therefore be related to hemolysis per se with a greater degree of confidence than would be the case with other diseases. Study of this group of patients also affords the opportunity to examine plasma gelsolin levels in patients before and after treatment, thereby excluding genetic causes of altered gelsolin levels.

MATERIALS AND METHODS

Patient samples. Samples of blood from children with acute Plasmodium falciparum malaria and 21- to 28-day convalescent sera were collected by Dr Kevin Marsh at the Outpatients Department, Medical Research Council Laboratories, Fajara, The Gambia. After parental consent was obtained, venous blood was collected and chloroquine treatment administered. These samples were collected in the rainy seasons of 1983 and 1984 when malaria transmission was high. Samples of blood from healthy adults and children, children with acute or subacute Plasmodium falciparum malaria, or patients with pneumonia were collected by Dr Sam Martin from villages in rural Nigeria. Patients with malaria or febrile seizures were...
between 2 and 6 years of age. All sera were stored at −20°C. *P. falciparum* malaria was diagnosed by light microscopic analysis of Giemsa-stained thick and thin blood films.

**Gelsolin assays.** Gelsolin concentrations of blood were determined by measuring gelsolin activity in blood samples as previously described.14 The assays are based on the ability of gelsolin to either accelerate the polymerization of actin monomers (nucleating assay) or sever actin filaments (severing activity).15,16 Changes in the fluorescence of pyrene-labeled actin were followed as a measure of either filament polymerization or filament severing. A standard curve was established each day by using a banked serum sample of known gelsolin concentration. Patient samples were assayed in triplicate and their gelsolin concentrations calculated from the component solutions, thus indicating that the hemoglobin present in the reaction mixture, which made it unlikely that variations in the ionic composition of the plasma samples affected the assay. To determine whether plasma-free hemoglobin affected the assay for gelsolin activity, purified plasma gelsolin was added to hemolyzed rabbit samples. The resulting mixtures had gelsolin activity as predicted from the component solutions, thus indicating that the hemoglobin present was diluted below a concentration where it could affect the assay.

**Identification of actin in plasma.** DNase I (Signa Chemical Co, St Louis) was coupled to CNBr-activated Sepharose 4B (Pharmacia Laboratories, Piscataway, NJ) at a concentration of 1 mg/mL of beads. One hundred-microliter samples of plasma and 100 μL of a solution containing 10 mmol/L Tris, 150 mmol/L NaCl, pH 7.4 (TBS) were added to 45 μL of a 50% (vol/vol) suspension of the beads. After tumbling for 90 minutes at 4°C, the beads were washed twice with TBS containing 1% Triton X-100 and twice with TBS without Triton. After the final wash, 30 μL of Laemmli's gel sample buffer17 was added, the beads placed in a boiling water bath for two minutes, and the mixture loaded onto 5% to 15% sodium dodecyl sulfate (SDS)–polyacrylamide gels for electrophoresis. Separated polypeptides were transferred to nitrocellulose sheets. Actin was identified by immunoblotting by the method of Towbin et al18 by using an antiactin antibody kindly provided by Drs Oman Skalbi and Giubio Gabbiani of the University of Geneva. Platelet actin was used as a positive control.

**Animal experiments.** Blood for hematocrit and gelsolin determinations (1 mL) was drawn from New Zealand white rabbits (3 to 4 kg) into heparinized syringes through a marginal ear vein and the plasma promptly separated. Hematocrits were determined by centrifugation of duplicate samples. Hemolysis was induced by one of two methods. One group of animals was injected with human serum albumin and then injected with human serum albumin (32 mg/mL). After the final wash, 30 μL of Laemmli's gel sample buffer17 was added, the beads placed in a boiling water bath for two minutes, and the mixture loaded onto 5% to 15% sodium dodecyl sulfate (SDS)–polyacrylamide gels for electrophoresis. Separated polypeptides were transferred to nitrocellulose sheets. Actin was identified by immunoblotting by the method of Towbin et al18 by using an antiaquactin antibody kindly provided by Drs Oman Skalbi and Giubio Gabbiani of the University of Geneva. Platelet actin was used as a positive control.

**RESULTS**

**Gelsolin levels in Nigerian children with malaria.** Plasma gelsolin concentrations were measured in samples from 18 acute malaria patients with two different functional assays for gelsolin activity. As shown in Table 1, the mean gelsolin level measured with the nucleating assay was 126 ± 45 μg/mL, and the mean gelsolin determined with the severing assay was 105 ± 32 (P > .05). The mean gelsolin level of the healthy subjects measured by the severing assay (265 ± 57 μg/mL) was lower than the gelsolin levels measured with the nucleating assay (367 ± 60 μg/mL), a finding consistent with circulating actin-gelsolin complexes.19 The mean gelsolin level of malaria patients was significantly lower than that of healthy subjects (P < .001 for either assay). For some groups, the gelsolin levels measured by the nucleating assay were significantly higher than those measured by the severing assay, a finding consistent with the presence of actin-gelsolin complexes. Because the exact conditions of blood drawing were not observed and this difference could have resulted from in vitro cell lysis, it was not pursued further in patient samples. Instead, comparisons were made by using either assay to compare gelsolin levels between patient groups.

The malaria patients could be divided into two groups: those with seizures and those without. Gelsolin levels were higher in patients with convulsions (Table 1). Gelsolin concentrations were measured in two other groups, healthy subjects (children and adults) and children of the same age who suffered from febrile seizures as a consequence of streptococcal pneumonia. Healthy subjects had mean gelsolin levels significantly greater than those of patients with either pneumonia or malaria as determined with either assay (P < .001).

**Changes in gelsolin levels with convalescence from malaria.** Serum samples from an additional 11 Gambian patients with malaria were examined. Samples were drawn at the time of admission with *P. falciparum* but before chloroquine administration. Convalescent samples were drawn approximately 3 weeks later. The mean levels of gelsolin in acute-phase samples were significantly lower than those in convalescent samples as measured by either assay (Table 2). When using the severing assay, the mean gelsolin concentration rose from 89 ± 68 to 201 ± 110 μg/mL (P < .001), whereas the nucleating assay showed a mean acute-phase level of 141 ± 133 μg/mL that rose to a mean level of 262 ± 160 μg/mL (P < .001). (One patient showed an apparent decline in gelsolin concentration after recovery as measured with the severing but not the nucleating assay. These values lie at the lower end of the standard curve.)

**Effects of experimental hemolysis.** We hypothesized that the low gelsolin levels seen were due to release of actin into the peripheral blood and the subsequent clearance of gelsolin-actin complexes. Consequently, hemolytic anemia was induced in rabbits to confirm that hemolysis could lower plasma gelsolin activity.

Phenylhydrazine was administered subcutaneously to cause oxidant-induced Heinz body hemolytic anemia, which

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**Table 1. Gelsolin Levels in Nigerian Malaria Patients and Controls**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nucleating Assay</th>
<th>Severing Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>11</td>
<td>367 ± 60</td>
</tr>
<tr>
<td>Pneumonia, seizures</td>
<td>8</td>
<td>116 ± 89</td>
</tr>
<tr>
<td>Malaria</td>
<td>18</td>
<td>126 ± 45</td>
</tr>
<tr>
<td>With seizures</td>
<td>9</td>
<td>160 ± 29</td>
</tr>
<tr>
<td>No seizures</td>
<td>9</td>
<td>92 ± 28</td>
</tr>
</tbody>
</table>

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developed over several days. As shown in Fig 1, plasma gelsolin activity changed little despite a large fall in the hematocrit value of the animals. This indicates that if accelerated clearance of plasma gelsolin occurred increased synthesis or mobilization from extravascular sites was sufficient to maintain normal plasma levels.

To introduce a larger amount of actin into the plasma more acutely, intravascular hemolysis was induced in rabbits by the injection of human serum. In contrast to the animals experiencing extravascular (oxidant-induced) hemolysis, animals undergoing acute intravascular hemolysis displayed a more rapid fall in hematocrit values with a simultaneous decline in plasma gelsolin activity, as shown in Fig 2. The gelsolin level as determined by the severing activity was lower than that determined by measuring the nucleating activity, a finding consistent with the formation of actin-gelsolin complexes.

Direct evidence for circulating actin in peripheral blood of animals undergoing acute hemolysis was obtained by precipitating actin from plasma with DNase-Sepharose beads and identifying the actin with antiactin antiserum. As shown in Fig 3, large amounts of actin were detected after the induction of hemolysis by the injection of human serum. Small amounts of actin were variably seen in samples drawn from healthy rabbits, thereby precluding a clear determination of whether actin is a normal constituent of plasma or induced during blood drawing. An acute drop in plasma

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**Table 2. Gelsolin Levels in Acutely III and Convalescent Malaria Patients From The Gambia**

<table>
<thead>
<tr>
<th></th>
<th>Severe assay (g/mL)</th>
<th>Nucleating assay (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Acute</td>
<td>Convalescent</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>226</td>
</tr>
<tr>
<td>2</td>
<td>240</td>
<td>300</td>
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<td>13</td>
</tr>
<tr>
<td>11</td>
<td>86</td>
<td>320</td>
</tr>
<tr>
<td>Mean</td>
<td>89 ± 68</td>
<td>201 ± 110</td>
</tr>
</tbody>
</table>

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**Fig 1.** Effects of phenylhydrazine on hematocrit and plasma gelsolin nucleating activity. Two rabbits were injected subcutaneously with phenylhydrazine on three successive days. Hemolysis was evident from the progressive fall in hematocrit values (triangles) without an immediate decline in the plasma gelsolin nucleating activity (circles).

**Fig 2.** Effect of acute intravascular hemolysis on plasma gelsolin activity. Two rabbits were injected IV with human serum, which caused a fall in the hematocrit value and gelsolin concentration of the plasma. The severing activity of gelsolin declined to a greater extent than did the nucleating activity, which is consistent with the formation of actin-gelsolin complexes.

**Fig 3.** Immunologic identification of actin in rabbit plasma. Plasma of a rabbit injected with human serum was incubated with DNase-Sepharose beads to precipitate actin. After washing, the polypeptides adhering to the beads were separated by electrophoresis on 10% to 20% SDS-polyacrylamide gels and transferred to nitrocellulose sheets and actin identified by immunoblotting. Platelet actin was run on each gel as a positive control (not shown). Samples were drawn at the following times after injection of serum: lane 1, zero time; lane 2, 17 minutes; lane 3, 38 minutes; lane 4, 90 minutes; lane 5, 280 minutes.
PLASMA GELSOLIN LEVELS IN MALARIA

Gelsolin concentrations can therefore be seen with acute intravascular hemolysis at a time when large amounts of actin are released into the plasma and is a likely explanation for the low gelsolin levels of acutely ill malaria patients and the subsequent elevation of gelsolin levels after treatment.

DISCUSSION

Gelsolin levels of patients with falciparum malaria were lower than those of healthy control subjects whose blood was drawn at the same time and stored under identical circumstances. Patients with streptococcal pneumonia and febrile seizures also had low gelsolin levels, in accord with our previous studies that have found depressed gelsolin levels in adults with bacterial pneumonia and adult respiratory distress syndrome. Low gelsolin levels are therefore not specific for malaria, hemolysis, or lung injury but may be expected in a variety of disease states characterized by cellular injury. Although the gelsolin levels of the control subjects were slightly higher than those of healthy white subjects studied in our laboratory (mean, 240 μg/mL), the gelsolin levels of the patients were clearly depressed when compared with either control group. Further support for the hypothesis that low gelsolin levels are related to the red cell destruction by P falciparum is found in the increase of gelsolin levels of patients after drug therapy.

Several facts support the idea that hemolysis caused depletion of gelsolin levels. First, hepatic involvement is not seen with falciparum malaria, and cells other than erythrocytes are rarely injured. Second, acute hemolytic anemia caused the gelsolin levels of rabbits to fall, whereas phenylhydrazine-induced subacute hemolysis caused anemia and only minimal depression of plasma gelsolin levels over a longer time span. The difference was likely due to the site and rate of hemolysis, being predominantly extravascular and slow for oxidant-induced hemolysis and intravascular and fast for serum-induced hemolysis.

The animals injected with human serum experienced an acute fall of approximately 50% in their hematocrit value. Estimating that 2 x 10^6 erythrocytes/μL were lysed and each erythrocyte contains 500,000 copies of actin (assuming rabbit and human erythrocytes are similar in this regard), the actin concentration of blood would be 1 μmol/L. Because the plasma concentration of gelsolin is 2 to 3 μmol/L, these rough calculations indicate that enough actin could be released during an acute hemolytic event to account for the experimental findings, and the calculations are consistent with the hypothesis that actin release from dying cells can lead to depletion of plasma gelsolin. (Premature platelet destruction in malaria might also contribute to depression of plasma gelsolin levels in malaria patients if platelet actin is exposed directly to plasma.)

In addition to gelsolin, plasma contains a second high-affinity actin-binding protein, the vitamin D-binding protein (DBP, also called Gc globulin). DBP binds actin monomers but not filaments and may serve an important role in clearing actin from the blood. Even though erythrocytes do not contain monomeric actin, changes in plasma levels of free DBP might be found in hemolytic states. The addition of F-actin to a large pool of plasma gelsolin leads to rapid depolymerization of filaments and the formation of 2:1 actin-gelsolin complexes. Because DBP can remove one actin molecule from such a 2:1 complex, DBP-actin complexes could form even if actin monomers were not introduced directly into the blood.

Two possible roles can be envisioned for the high-affinity actin-binding proteins of blood, gelsolin, and DBP. First, they may function primarily to depolymerize actin filaments released into the extracellular space after tissue injury. Left unshortened, long actin filaments might have deleterious effects such as altering the rheological properties of blood by raising its viscosity. Actin filaments could alter patterns of blood flow and/or increase the contact time of activated blood cells with the vessel wall, thereby potentiating tissue injury. The demonstration that long actin filaments can affect fibrin clot formation while gelsolin-shortened filaments do not, suggests that long actin filaments may be deleterious in other ways and indicates a manner in which gelsolin may protect against the harmful effects of extracellular actin.

Second, the DBP-gelsolin system may function as a disposal mechanism to clear actin from the blood. Because actin is the major cellular protein of nucleated cells (as well as platelets), large amounts of the protein would be expected to enter the plasma under both physiologic and pathologic conditions. Given the large amount of actin in the body as a whole, efficient clearance of actin may be of significance in itself.

Thus, gelsolin and DBP have complementary properties and constitute a system of plasma proteins that work together to shorten and clear the cytoplasmic proteins actin. Because subjects congenitally deficient in these proteins have not been described, the physiologic importance of this system must be derived from analyses of subjects with different diseases. The studies reported here are useful therefore in developing the understanding of the roles of these proteins in more complex disease states.

Although other unexplored factors (such as malnutrition) may play a role in causing the low gelsolin levels seen in patients with malaria, it seems likely that gelsolin levels were depressed after hemolysis and that similar results might be expected in patients experiencing acute hemolytic events such as after major blood transfusion reactions, the hemolytic crisis of sickle cell disease, or acute hemolysis resulting from glucose-6-phosphate dehydrogenase deficiency.

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