**PLASMODIUM FALCIPARUM MALARIA IN SPLENECTOMIZED PATIENTS: TWO CASE REPORTS IN FRENCH GUIANA AND A LITERATURE REVIEW**

MAGALIE DEMAR, ERIC LEGRAND, DIDIER HOMMEL, PHILIPPE ESTERRE, AND BERNARD CARME

Parasitology-Mycology Laboratory, French West Indies and Guiana Faculty of Medicine and Cayenne Central Hospital (EA3593 Team), Cayenne, French Guiana; National Reference Center on Plasmodium Chemo-resistance in the French West Indies and French Guiana, Institut Pasteur de Guyane, Cayenne, French Guiana; Intensive Care Unit, Cayenne Central Hospital, Cayenne, French Guiana

Abstract. Some of the immunologic mechanisms involved in malaria physiopathology remain unclear. In animals, the spleen seems to play a key role in protecting the host against malaria. However, little is known about the effect of spleen dysfunction on human malaria. We report two severe cases of *Plasmodium falciparum* infection with unusual clinical and parasitologic features in two splenectomized men living in French Guiana. The peripheral blood of these cases showed hyperparasitemia, with high proportions of mature parasites and leukocytes with malaria pigment. Despite appropriate treatment and adequate absorption, hyperparasitemia persisted. Parasite clearance was delayed and one patient died. Only the patient who died had the merozoite surface protein 1 allele B-K1 and the varD gene genotype, which is considered to be a probable parasite virulence factor. These uncommon cases differ from most of those described in the literature, illustrating the complexity of the mechanisms underlying the protective function of the spleen in human malaria.

**INTRODUCTION**

Antimalarial immunity involves the humoral (IgG and IgM antibodies) and cellular (essentially involving the spleen) immune responses. The role of the spleen in the removal of intra-erythrocytic parasites has been demonstrated in numerous animal models, e.g., splenectomy increases susceptibility to and severity of malaria infection. Conversely, little is known about the effect of splenectomy on human malaria. Only a small number of African, Asian, and American cases have been described in the official literature. On the basis of four Thai case reports, Looareesuwan and others concluded that the spleen may not be essential for the processes leading to parasite clearance in partially immune patients.

The rare clinical and parasitologic features of *Plasmodium falciparum* infections that we report in two immune, splenectomized men living in French Guiana do not support this conclusion.

**MATERIALS AND METHODS**

**Study site.** Malaria is a major public health problem in French Guiana, a French province situated in the Amazon basin in South America. It has a population of 156,790 inhabitants according to official report in March 1999. Between 3,000 and 5,000 proven cases occur each year, mostly along the Maroni and Oyapock Rivers. Although the transmission rate is moderate in these two inland regions compared with African countries, transmission occurs continually, leading to the development of premunition in adults. The two patients we studied were hospitalized in the intensive care unit of Cayenne Hospital. They came from regions along the lower Maroni and Oyapock Rivers.

**Parasitologic features.** Parasitemia was determined daily using anticoagulated venous blood. Asexual parasites in blood smears were counted and classified into five stages according to the modified method of Silamut and White: tiny, small, and large ring forms, mature trophozoites, and schizonts. We expressed the presence of malaria pigment in neutrophils and monocytes as the percentage of pigment-containing leukocytes. Analysis of parasite morphology showed that drug-affected parasites had pyknotic nuclei and a shrunken cytoplasm. The parasite clearance rates, PC_{50} and PC_{90}, are defined as the times taken from the start of antimalarial treatment until the asexual malaria parasite count decreased to 50% and 96%, respectively, of the admission value.

**Molecular features.** We attempted to detect the highly polymorphic merozoite surface protein 1 (msp-1) block 2 gene and the Duffy binding-like γ region of the varD virulence gene using anticoagulated blood from patient 2 and blood smears collected from patient 1 on admission. We used the polymerase chain reaction (PCR) protocols previously described by Ariey and others. The PCR products (msp-1 and varD) were analyzed by agarose gel electrophoresis and staining with ethidium bromide.

**RESULTS**

**Case 1.** A 48-year-old Amerindian man was hospitalized with a five-day history of febrile icterus. In 1993, he had undergone a splenectomy following chronic visceral malaria. Upon admission, he presented hyperthermia (temperature = 38.4°C), arterial hypotension (blood pressure = 90/60 mm Hg), macroscopic hematuria, and cutaneous-macular icterus. He displayed thrombocytopenia (12 × 10^9/L), functional renal insufficiency (creatinine = 105 μmol/L and urea = 23.5 mmol/L), and a high level of total bilirubin (233 μmol/L). There was no evidence of previous antimalarial treatment, underlying disorders, or immunodeficiency diseases such as infections with human immunodeficiency virus or human T cell lymphotrophic virus. Examination of blood films showed asexual forms of *P. falciparum* in 15% of the erythrocytes in the peripheral blood. Although ring forms predominated, mature trophozoites and schizonts were also present (Table 1). No sexual parasites were found. Malaria pigment was visible in 7% of peripheral blood leukocytes. The erythrocytes showed anisopoikilocytosis with numerous Howell-Jolly bodies. Indirect immunofluorescence (Immunofluorescence *Plasmodium* spot-IF with normal values < 1:80; Biomérieux, Lyon, France) showed that the titer of antibodies to *P. falciparum* was 1:1,600. The direct antiglobulin test (DAT) result...
was positive for IgG. The *in vitro* antimalarial sensitivity assay was unsuccessful because the parasite did not grow. Parasite genome analysis showed the B-K1 allele of the *msp-1* gene associated with the *varD* gene. The patient initially received an intravenous loading dose of quinine formiate (Quinoforme®; Sanofi-Synthélabo, Bagneux, France) (16 mg/kg over a four-hour period). He then received 8 mg/kg of intravenous quinine formiate three times per day plus 3 mg/kg of intravenous tetracycline (Vibraveineuse®; Pfizer, Angers, France) twice a day for six days. Oral mefloquine (Lariam®; F. Hoffmann LaRoche, Basel, Switzerland) was given on the sixth day (24 mg/kg over 24 hours). The parasitemia stabilized 48 hours after initiation of treatment and then slowly decreased (Figure 1). The PC₅₀ and PC₉₆ were 60 hours and 84 hours, respectively. Drug-affected parasites were detected from the second day onwards. The daily plasma quinine concentrations measured by fluorimetry were satisfactory, ranging from 6.38 to 9.45 mg/L (therapeutic range = 8–20 mg/L). The patient became afebrile 55 hours after admission, but his clinical state got progressively worse and he died on day 9 following massive bleeding of the digestive tract with disseminated intravascular coagulation three days after the end of the treatment. Blood smears remained positive for *P. falciparum* trophozoites (0.008%).

**Case 2.** A 29-year-old Maroon man was transferred to Cayenne Hospital with a *P. falciparum* infection. In 1988, he underwent splenectomy following abdominal trauma in a traffic accident. He had had several uncomplicated malaria attacks. Forty-five days before being hospitalized, he had experienced febrile digestive disorders for which he treated himself successively with halofantrine (Halfan®; GlaxoSmithKline, Nanterre, France), then with five tablets per day of chloroquine (Nivaquine®; Aventis, Paris, France) for five days without any real clinical improvement. Upon admission, the patient was febrile (temperature = 39.2°C), with arterial hypotension (blood pressure = 89/56 mm of Hg) and poor clinical condition. Biologic disorders such as thrombocytopenia (32,000/mm³), organic renal insufficiency (creatinine = 445 μmol/L, urea = 26.8 mmol/L), and hyperleukocytosis (39800/mm³) were detected. Thirty percent of the red blood cells were parasitized with asexual forms of *P. falciparum*. All the stages were present, with a high proportion of mature forms, especially schizonts (Table 1 and Figure 2). Malaria pigment was detected in 6.7% of the peripheral blood leukocytes. The erythrocytes displayed anisopoikilocytosis with some Howell-Jolly bodies. The titer of antibodies to *P. falciparum* was 1:400. The DAT result was positive for IgG and the complement C3. The *in vitro* antimalarial sensitivity assay was un-

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Parasite density (asexual forms, %)</th>
<th>Parasite distribution (%) asexual forms of <em>Plasmodium falciparum</em></th>
<th>Pigment-containing leukocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Tiny rings</td>
<td>Small rings</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>Patient 2</td>
<td>30</td>
<td>10</td>
</tr>
</tbody>
</table>

---

**FIGURE 1.** Parasite density and central body temperature during treatment of *Plasmodium falciparum* malaria in two asplenic men. The dark arrows indicate the beginning of the treatment and the gray arrows indicate the beginning of the decrease in parasite load. The horizontal dotted line in the upper graph is the threshold of hyperthermia.

**FIGURE 2.** Thin blood film from patient 2 showing all stages of the asexual forms of *Plasmodium falciparum* with a high proportion of mature schizonts.
successful because the parasite did not grow. The parasite had the K1 allele of the msp-1 gene, but not the varD gene. The patient initially received an intravenous loading dose of quinine formiate (Quinoforme®) (16.33 mg/kg over a four-hour period). He then received 8 mg/kg of intravenous quinine formiate three times a day plus 3 mg/kg of intravenous tetracycline (Vibravineuse®) twice a day for seven days. Treatment was continued with oral mefloquine (Lariam®), introduced on day 7. Based on the daily plasma quinine concentrations measured by fluorometry, the molecule was correctly absorbed, with values ranging from 22.8 to 37.8 μmol/L. Parasitemia remained fairly constant for the first 65 hours after initiation of treatment. However, it then decreased gradually over a prolonged period (Figure 1). The $PC_{50}$ and $PC_{90}$ were 102 and 127 hours, respectively. Drug-affected forms were detected from the first day onwards. The patient became afebrile 86 hours after admission. Slides were negative for malaria parasites on day 11 and the patient was discharged on day 14.

**DISCUSSION**

Although laboratory experiments using animal models have confirmed the importance of the spleen in the host’s defense against *Plasmodium*, the exact mechanisms of its protective effect remain unclear. Interactions between parasite antigens on the surface of the infected erythrocytes and receptor molecules on endothelial cells allow parasitized red blood cells (PRBCs) (parasitized with mature parasite stages) to adhere to capillary and postcapillary venular endothelial cells. This parasite sequestration phenomenon usually limits the observable parasitemia to ring forms and gametocytes. This is not found in splenectomized primates, suggesting that the spleen removes mature *P. falciparum* stages from the peripheral blood by modulating the surface antigens involved in cytoadhesion and thus parasite sequestration. Furthermore, the spleen enhances the removal of infected red cells with reduced deformability such as PRBCs and those coated with antibodies.

The clinical and parasitologic features of malaria in the two splenectomized patients highlight these events. However, they contradict some previous hypotheses suggesting that the role of the spleen in cytoadhesion and parasite clearance in acute human malarial infection depends on immune status. Indeed, both of the patients were immune with a moderate (patient 2) or high (patient 1) level of antibodies to *P. falciparum*. Although computed tomography scans of the abdomen and technetium scans were not performed, there were other indirect signs confirming that the patients had undergone splenectomy: the abdominal scar, abnormal red blood cell morphology, and Howell-Jolly bodies in the blood smears. Both patients had severe malaria according to the clinical and biologic criteria defined by the World Health Organization (WHO). Their peripheral blood displayed a high level of parasitemia with all developmental stages of the parasite, which resembled the asplenic simian model of malaria. They had unusually high proportions of more than 26-hour parasitologic stages, especially schizonts: 75% in patient 2 and 4% in patient 1. The persistence of PBRCs in the peripheral blood indicates that sequestration and splenic clearance may not have occurred.

Similar parasitologic features were previously described in splenectomized but nonimmune patients. Furthermore, Looareesuwan and others reported uncomplicated *P. falciparum* infection in two asplenic and partially immune Thai patients who, unlike our two cases, presented signs of *in vivo* sequestration, normal parasite clearance, and normal humoral and cellular immune responses to blood stage antigens. David and others observed the reverse action of immune serum on *in vivo* sequestration in intact animals. The absence of a spleen may slow down the clearance of infected red blood cells sensitized with IgG antibodies. Thus, PRBCs displaying IgG on their surface membranes were actively and intensively phagocytosed by monocytes and neutrophils in the peripheral blood from both patients, with a high proportion of pigment-containing leukocytes. In addition, the results of the DATs were positive, showing that IgG and complement factor C3d were present on erythrocytes.

Despite appropriate treatment with drugs recommended for such clinical cases and adequate absorption, both cases progressed abnormally; hyperparasitemia persisted for 48 hours after initiation of treatment and parasite clearance was delayed (no clearance for patient 1 and 12 days for patient 2). Although the parasite density at 48 hours fulfills the conventional WHO criteria for R3 drug resistance, some parasites appeared to be drug-affected and are likely to have been dead. Indeed, resistance seems unlikely because no cases of *in vivo* resistance have been reported with this association (quinine plus vibramycin) in French Guiana. These findings are consistent with those previously reported, thus confirming the inaccuracy of the official criteria of antimalarial drug resistance in asplenic patients.

These uncommon observations suggest that regardless of immune status, clinical and parasitologic aspects in asplenic humans may be the result of a complex combination of host factors such as host susceptibility and/or virulence of the strain. Only the patient who died had the B-K1 allele plus the var D gene. This is in agreement with a previous study in French Guiana, which showed that this genotype is consistently present in most severe clinical cases. Furthermore, these two patients belonged to two distinct ethnic groups and may have had different genotypes conferring different levels of receptivity to the *Plasmodium* parasite. This has not been explored.

It is currently difficult to know whether these atypical parasitologic features were due to host susceptibility or to the virulence of the strain, but these observations highlight the need for further studies on the pathophysiology of malaria infection in splenectomized patients.

Received November 4, 2003. Accepted for publication April 5, 2004.

Acknowledgments: We thank Anne Lavergne for aligning the msp-1 gene and Ruth Mathar-Heraud for evaluating parasitemia and parasite distribution and counting the leukocytes with malaria pigment.

Financial support: This work was supported by the Délégation Générale du Réseau des Instituts Pasteur and Instituts Associées.

Authors’ addresses: Magalie Demar and Bernard Carme, Parasitology-Mycology Laboratory, French West Indies and Guiana Faculty of Medicine and Cayenne Central Hospital (EA3593 Team), BP 6006, 97306 Cayenne, French Guiana, Telephone/Fax: 594-594-39-53-09 or 594-594-28-72-63, E-mails: mdemar@yahoo.com and b.carme@plus.fr, Eric Legrand and Philippe Esterré, National Reference Center on Plasmodium Chemoresistance in the French West Indies and French Guiana, Institut Pasteur de Guyane, Cayenne,
REFERENCES


