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Invasion of Erythroblasts by *Pasmodium vivax*: A New Mechanism Contributing to Malarial Anemia

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ABSTRACT

Severe malarial anemia causes considerable mortality and morbidity in endemic areas. Possible mechanisms underlying the anemia include lysis of parasitized and nonparasitized red cells as well as parasite product-mediated effects on erythropoiesis. The latter include suppression of erythropoiesis, dyserythropoiesis, and ineffective erythropoiesis. Present transmission electron microscope data in two cases of *Pasmodium vivax* malaria show a hitherto undescribed mechanism contributing to malarial anemia, namely, infection of erythroblasts by parasites and their subsequent degradation. No parasites were detected in the peripheral blood but parasites were found in the bone marrow. These findings emphasise the value of bone marrow examination in the diagnosis and eradication of malaria.

Keywords: bone marrow, erythroblasts, malarial anemia, mechanism

Malaria is a mosquito-borne infectious disease resulting from infection of hepatocytes and erythrocytes by protozoan parasites of the genus *Plasmodium*. Anemia is a key feature and is found both in acute malaria with high parasitemia and chronic malaria with low parasitemia. The characteristic periodic symptoms found in acute malaria accompany the release of merozoites from liver cells and erythrocytes. Most of the research on the pathogenesis of malarial anemia has been done on *P. falciparum* malaria. The data show that the mechanisms

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underlying the anemia are complex, involving the destruction of both parasitized and nonparasitized red cells and a failure of the bone marrow to compensate for the accelerated removal of erythrocytes by an adequate increase in effective erythropoiesis. Whereas destruction of red cells plays an important role in the pathogenesis of the anemia in acute malaria, the anemia of patients with low-level parasitemia is not primarily due to erythrocyte destruction and is largely caused by suppression of erythropoiesis, dyserythropoiesis, and ineffective erythropoiesis. The disordered eythropoiesis is thought to result from direct or indirect effects on erythropoiesis of parasite products released during schizogony, such as malaria pigment (hemozoin), heat-stable soluble antigen, GPI anchor, and various exoantigens, such as ag1 and ag7.

Infection of erythroblasts by any species of malarial parasite has not been reported previously. Here we report transmission electron microscope data on 2 patients with chronic *P. vivax* malaria, showing parasites within erythroblasts. This finding suggests that infection and destruction of erythroblasts by parasites may be another mechanism underlying the anaemia of this clinical form of malaria.

CASE REPORT

Case 1

A 42-year-old male from Henan province presented with a 3-month history of anemia and jaundice, and was admitted to our hospital in July 2006. From beginning, he had no typical malaria symptoms, such as periodical fever, chills, rigors, or sweats. His physical examination revealed slight splenomegaly, confirmed by ultrasound examination. Wright's-stained blood smears had not shown malarial parasites. Bone marrow smears contained red cells with *Plasmodium vivax* at different stages of development such as ring forms, trophozoites, and early and late schizonts (Figures 1, 2 and 3). Erythroblasts showed various abnormalities, including megaloblastic change, condensed and double nuclei, palely stained area in cytoplasm, and cytolysis. The ratio of granulocyte

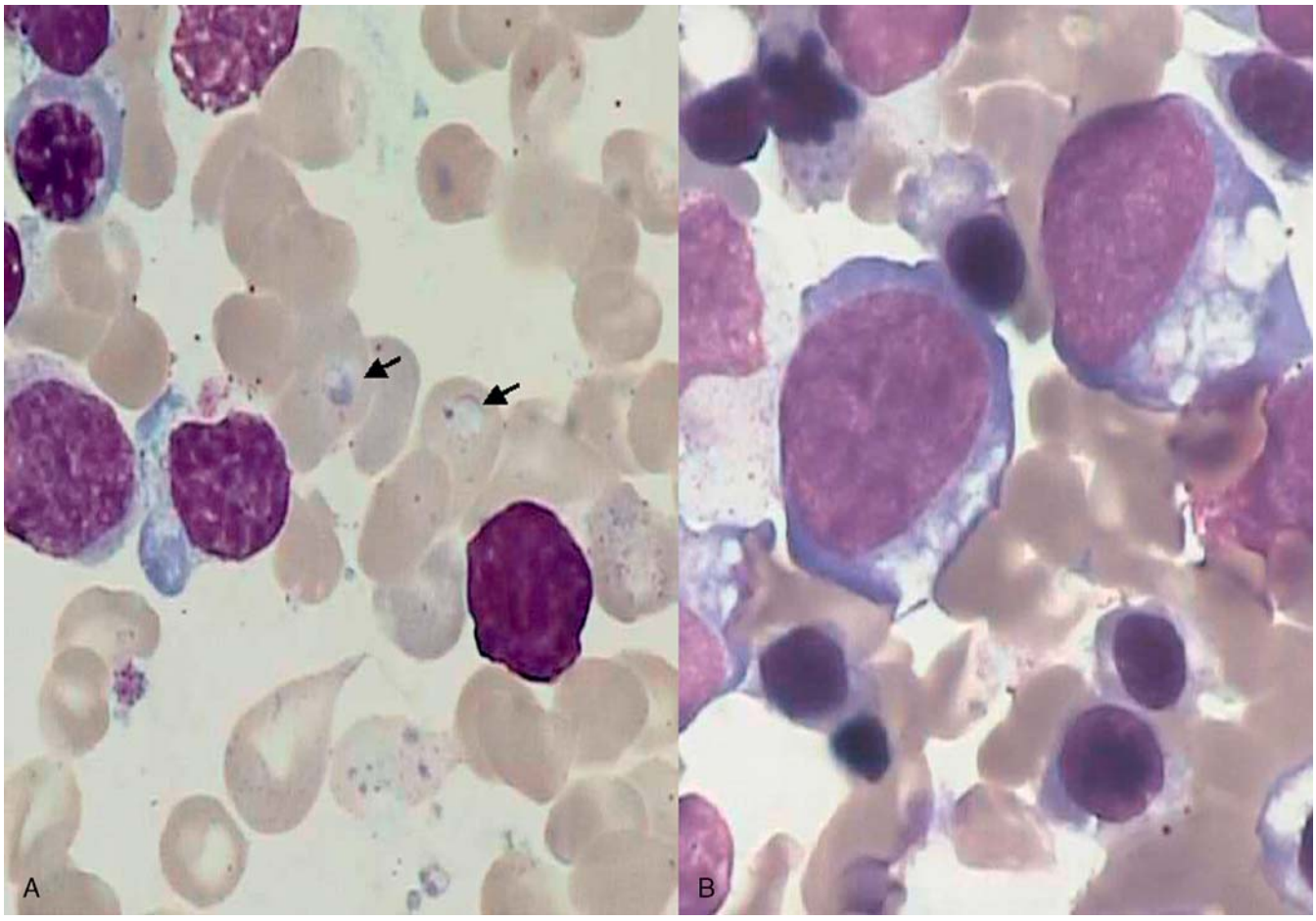

Figure 1. Wright's-stained bone marrow smears. (A) mature erythrocytes containing malaria parasites at various stages of maturation: ring forms (short arrows), trophozoites, early and late Schizonts (arrow); (B) basophilic erythroblasts or megaloblastes with unstained areas within the cytoplasm, $\times 100$.

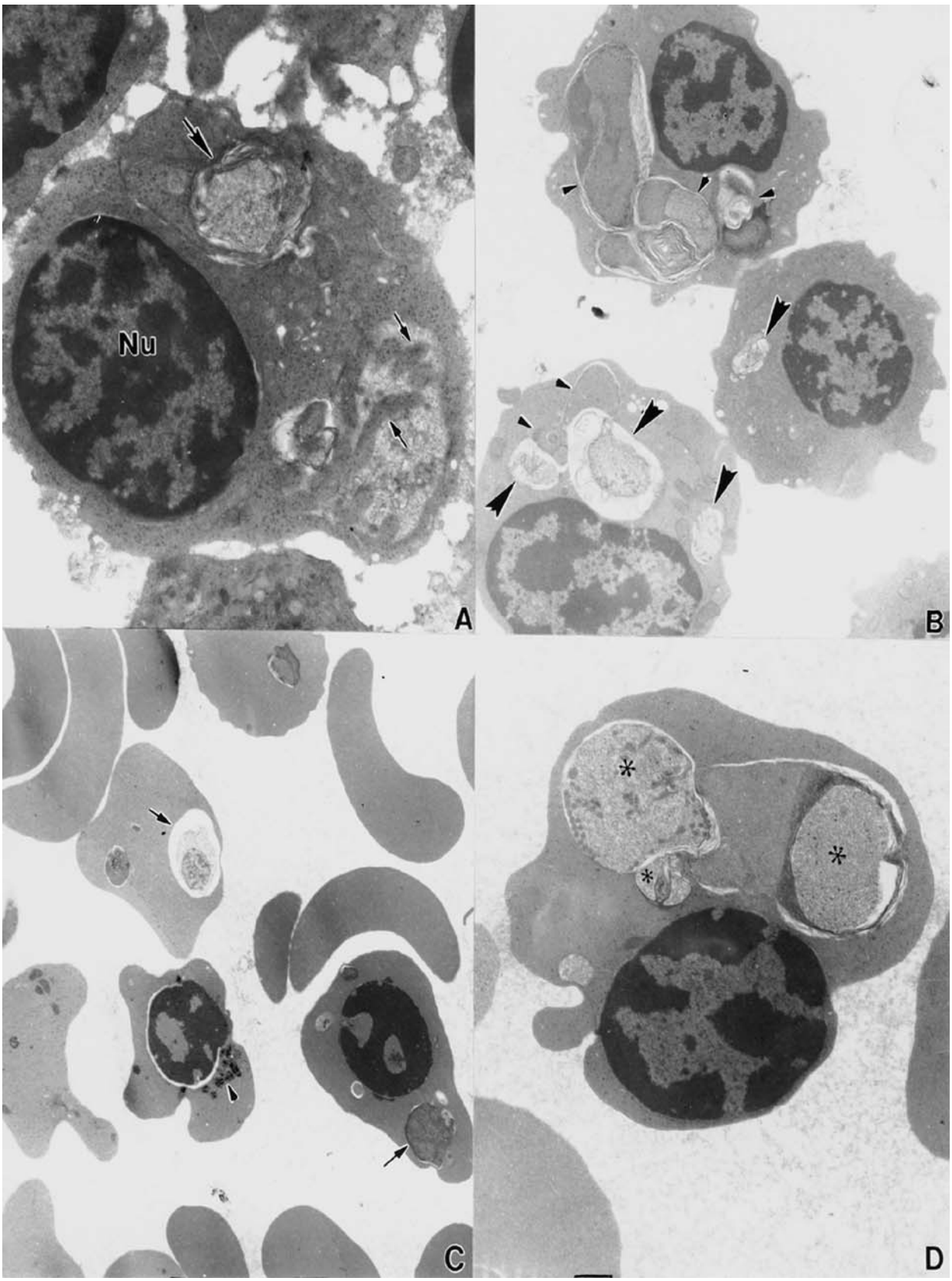

Figure 2. (A) Erythroblast containing schizonts with developing merozoites (small arrows), ×6000; (B) polychromatophilic erythroblast with various trophczoites (arrowheads), ×3000; (C) damaged orthochromatic erythroblasts with trophczoite (arrows), and sideroblast (arrowheads), ×3000; (D) orthochromatic erythroblast containing trophczoites (asterisks), ×5000.

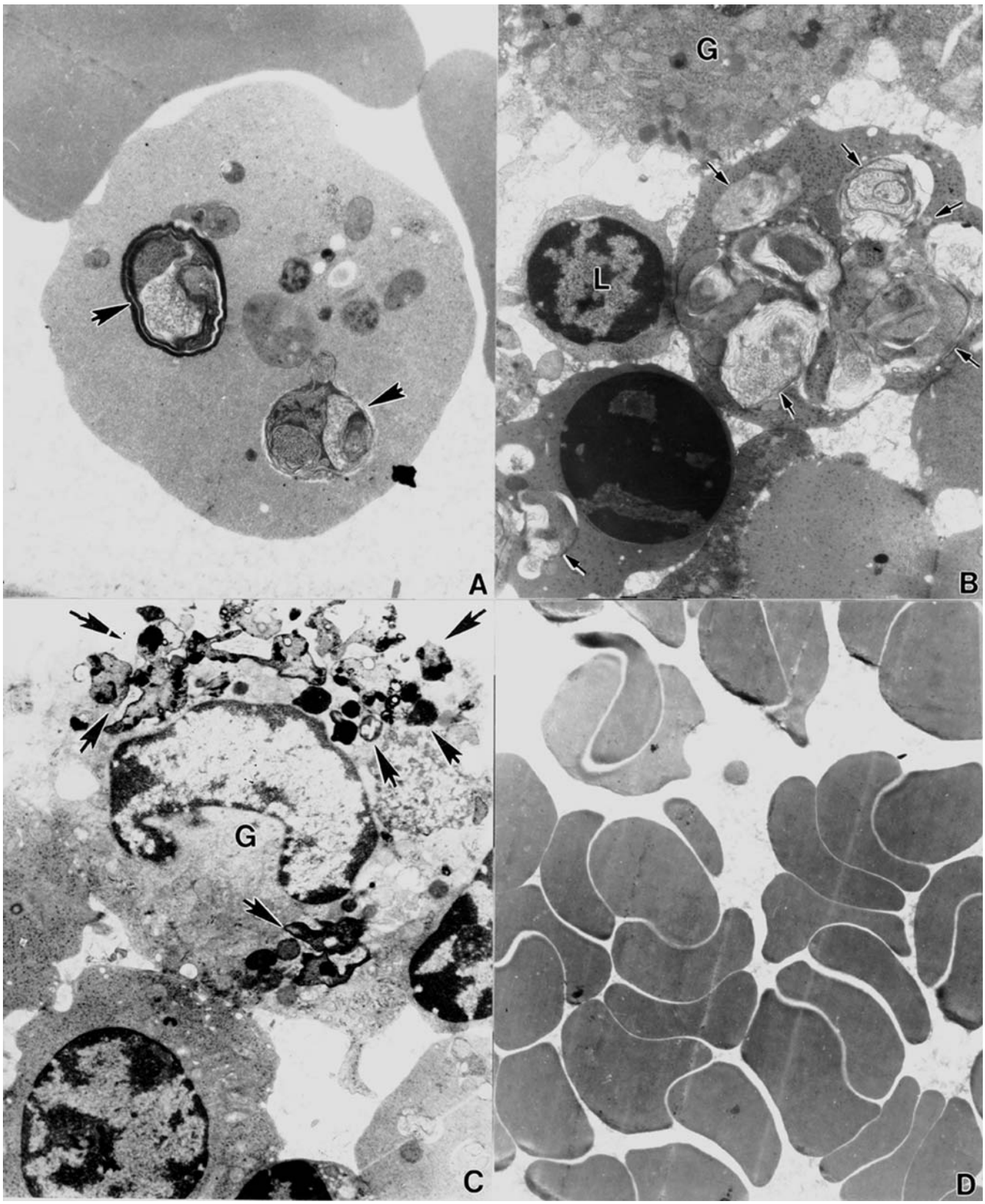

Figure 3. (A) Non-nucleated erythroid cell with various trophczoites, ×8000; (B) an orthochromatic erythroblast and erythroid cell containing trophczoites, ×6000; (C) granulocyte or macrophage containing hemozoin, ×5000; (D) mature erythrocytes without malaria parasites, ×3000.

Table 1. Relevant laboratory examinations of two cases.

Cases 1			
WBC	3.21×109	TBIL	40.21 (1.7–26.5 μmol/L)
RBC	3.79×1012/L	DBIL	25.33 (1–10 μmol/L)
HB	110 g/L	IBIL	14.88 (0–23.3 μmol/L)
PLT	196×109/L	SHT	–
MCV	85 fL	Ham test	–
RET	0.7	Coombs	–
CD55	–	IMT	normal
CD59	–		
Cases 2			
WBC	4.25×109	TBIL	30.23 (1.7–26.5 μmol/L)
RBC	3.11×1012/L	DBIL	12.38 (1–10 μmol/L)
HB	75 g/L	IBIL	18.85 (0–23.3 μmol/L)
PLT	135×109/L	Serum iron	33.3 (9.95–28.64 μmol/L)
MCV	98(80–94 fL)	UIBC	7.80 (29.54–63.54 μmol/L)
RET	0.8	Transferrin saturation	0.81 (0.25–0.5)
Sideroblasts	90%	Serum ferritin	311.0 (12–120 μg/L)
		Folic acid	8.96 (8.5–29.5 μmol/L)
		Vitamin B_{12}	268 (133–800 pmol/L)

series cells to erythroblasts (G/E) was 2:1–3:1. Other results of the laboratory examination are listed in Table 1.

Case 2

A 35-year-old female from Hebei province was admitted to our hospital in December 2007 with a 10-year history of anemia. She had not experienced typical malaria symptoms, such as periodical fever, headache, chills, rigors, and abdominal pain. Physical and ultrasound examination showed no enlargement of the spleen and liver. Her hemoglobin had fluctuated between 70 and 80 g/L over 10 years and she had previously been suspected to have a myelodysplastic syndrome (refractory anemia). Blood smear examination (Giemsa stain) had not shown plasmodium. Bone marrow smears exhibited *Plasmodium vivax* in occasional erythrocytes. Erythroblast abnormalities included megaloblastic change, abnormally condensed nuclei, binuclearity, and pale areas in the cytoplasm. Other results of the laboratory examination are listed in Table 1.

TRANSMISSION ELECTRON MICROSCOPY

The bone marrow aspirate (BMA) was prepared according to our previous work [1]. Briefly, isolated nucleated cells ($\times 10^6$ cells) from 3–5 mL of anticoagulated BMA were fixed in 2% glutaraldehyde of PBS (pH 7.4) for 1 h, postfixed in 1% osmium tetroxide, dehydrated in graded alcohol, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and then examined with a Hitachi TEM (H-600).

Ultrastructural characteristics of the nucleated cells of different lineages were similar in the two patients. Erythroblasts showed abnormalities such as megaloblastic change, binuclearity, apoptosis, and lysis. Proerythroblasts, basophilic erythroblasts, early polychromatic erythroblasts, orthochromatic erythroblasts, nonnucleated profiles of erythroid cells, and reticulocytes contained malaria parasites. Parasites at different developmental stages, including ring forms, trophozoites, early and late schizonts, as well as gametocyte-like forms, were found, usually within different erythroblasts (Figures 2 and 3). Granulocytes usually showed reactive features, with more processes on the cell surface and focal cytoplasmic necrosis. Some macrophages and granulocytes contained degenerating and vestigial plasmodium parasites, matching with the descripion of hemozoin in the literature [2] (Figure 3C). Eosinophil granulocytes and plasma cells were often found.

DISCUSSION

Malaria is widely distributed in areas of southeast Asia. *P. vivax* and *P. falciparum* infections are most prevalent in the southeast of China [3]. Schizogony takes place in capillaries of internal organs, where infected red cells tend to adhere to small vessels that become plugged. This often produces serious anemia, and in the case of *falciparum* malaria, potentially fatal complications such as cerebral malaria, renal failure, acute respiratory distress syndrome as well as liver

dysfunction. Distinctive symptoms of *vivax* and *falciparum* malaria are periodic chills, rigors, fever, and sweats besides the common complaints of headache, abdominal pain, and vomiting [4].

Malaria diagnosis is usually based on clinical symptoms and examination of Giemsa-stained blood smears, the latter being the gold standard. Recently, immunological and PCR methods have been shown to be of value and are now being used. Whereas in most patients light microscopy reveals parasites within erythrocytes in peripheral blood smears, in some cases few or no parasites are detected. The latter present with anemia rather than typical malaria symptoms and are sometimes described as having asymptomatic malarial infection [5].

Our two patients presented with persistent anemia, slight jaundice, and no other symptoms of malaria. Parasites were not detected in the peripheral blood by light microscopy, and the diagnosis was not established until parasites were detected in the bone marrow by light and electron microscopy in our hospital. Therefore, both patients fell into the category of asymptomatic malarial infection.

Anemia is a common finding not only in acute malaria with high parasitaemia but also in asymptomatic infection with low parasitemia [6]. In the latter, suppression of erythropoiesis (reduced total erythropoiesis), dyserythropoiesis (disordered erythropoiesis with morphological abnormalities in erythroblasts), and ineffective erythropoiesis (increased rate of apoptosis and phagocytosis of erythroblasts) are more important than erythrocyte destruction in the pathogenesis of the anemia [7]. Parasite products such as hemozoin may indirectly impair erythropoiesis by stimulating the release of cytokines such as IFNγ, TNF-α, IL-2, and IL-10 from T-cells and TNF-α and IL-1 from macrophages; some of the released cytokines, especially IFNγ and TNF-α, or an imbalance between pro-inflammatory and anti-inflammatory cytokines may cause dyserythropoiesis [8–11]. Parasite products may also reduce the formation of erythroid progenitors either by direct effects or via suppression of erythropoietin production [12]. Kurtzhals and Helleberg thought that severe anemia of patients with asymptomatic infection might result from effects of parasitized red cells sequestered in the bone marrow, though parasites were undetectable in peripheral blood [13, 14].

Light and electron microscope studies of the bone marrow in 9 Thai adults with anemia due to *P. vivax* malaria have shown dyserythropoiesis and erythroblast degradation by macrophages, as well as macrophage hyperplasia, plasmacytosis, and increased eosinophils [15]. Our studies not only confirmed these findings in 2 cases of *P. vivax* malaria, but also demonstrated

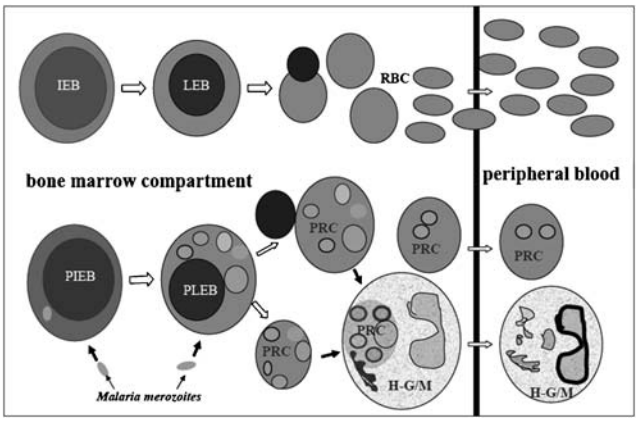

Figure 4. Mechanism of low-level parasitemia. In bone marrow, plasmodium are mainly infesting and parasitized in intermediate and late erythroblasts(PIEB and PLEB). Most of parasitized RBCs (PRC) are phagocytized by macrophages or granulocytes (H-G/M) and fewer PRCs are released into peripheral blood.

parasites at various stages of maturation within intermediate and late erythroblasts (PIEB and PLEB) and mature red cells. Granulocytes and monocytes contained degenerating erythrocytes and hemozoin. In a large number of marrow aspirates from children with chronic *P. falciparum* malaria studied previously, parasites were not seen within erythroblasts [16, 17]. Our observations suggest that in some cases of *P. vivax* malaria suppression of erythropoiesis might result from plasmodium parasitizing and destroying erythroblasts. Such cases may have few or no parasites detectable in the peripheral blood by light microscopy (Figure 4).

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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