Exflagellation of Malarial Parasites in Human Peripheral Blood

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Exflagellation of microgametocytes in malarial parasites is associated with the life cycle in the mosquito. However, it is not found with the usual developmental phases in humans. This report describes two case reports where exflagellation of microgametocytes was observed in vitro in patients documented to have *Plasmodium vivax* malaria.

Exflagellation of the microgametocyte in the life cycle of malarial parasites occurs in the stomach of mosquitoes following an infective blood meal. In 1897, exflagellation was observed by MacCallum in blood smears obtained from a patient with *Plasmodium falciparum* infection (3). The conditions for in vitro exflagellation have been described, and the phenomenon has been observed in smear preparations containing the chicken malarial parasite, *Plasmodium gallinaceum* (1), as well as in other cases of *P. falciparum* human infection (2). Because exflagellation is not associated with the usual developmental phases of plasmodia in humans, there may be confusion about the identity of the flagellar form unless this phenomenon is recognized. We have observed exflagellation in the peripheral blood films of two patients with *Plasmodium vivax* malaria.

Case report I. The first patient, a 43-year-old Asian Indian, had been traveling in a region of India where malaria has reemerged. Eight days after his arrival in the United States, he had the sudden onset of fever, chills, and a frontal headache. Four days later at an emergency room, he was afebrile and the physical examination was unremarkable. The hematocrit was 44%, and the leukocyte count was 4,100 per mm³ with 44% segmented neutrophils, 11% band neutrophils, 42% lymphocytes, and 3% monocytes. Examination of the peripheral blood film for malaria revealed a number of ring forms and amoeboid trophozoites consistent with *P. vivax*. In addition, several filamentous microgametes were seen outside the erythrocytes. The thin, undulating structures measured approximately 15 μm in length and contained a dark, cigar-shaped nucleus after staining with either Wright or Giemsa stain (Fig. 1). The diagnosis of *P.*

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vivax infection with exflagellation of microgametes was confirmed at the Center for Disease Control. The patient received chloroquine and primaquine therapy and recovered uneventfully.

Case report II. The second patient, a 32-year-old truck driver from Chicago, Ill., had emigrated from Poland 1 year prior to hospital admission. He had traveled widely in the continental United States and in Europe. While driving his truck, he experienced severe chills, malaise, abdominal pain, and fever to 106°F (41°C). He was admitted to a local hospital and was found to be febrile (102°F; 38.8°C) but without nuchal rigidity or respiratory distress. The admission hematocrit was 33%, and the leukocyte count was 3,300 per mm³ with 47% segmented neutrophils, 24% band neutrophils, 26% lymphocytes, 1% monocytes, and 2% eosinophils.

Peripheral blood films demonstrated infection of immature erythrocytes, Schüffner's dots, and the presence of trophozoite ring forms compatible with *P. vivax*. In addition, there were several exflagellated microgametes on the blood smears. The patient was treated with supportive therapy and chloroquine and discharged for further follow-up by his family physician.

Laboratory studies. Attempts to get additional blood samples for evaluation of exflagellation in vitro were unsuccessful. Blood samples were collected from 10 normal volunteers into tubes that contained ethylenediaminetetraacetic acid. In addition, random tubes that had been submitted to the Hematology Laboratory were tested after the hematological studies were completed. The pH was tested in tubes that sat in a rack with the stopper in place or removed and in tubes that were rotated (Adams rotator) for 4 h with the stopper in place. In each instance, the initial pH was below 7.4, and over a period of 4 to 6 h, the pH decreased slightly. The plasma in 10 tubes that stood unstopped overnight reached pH 7.9 ± 0.1, but the pH of the
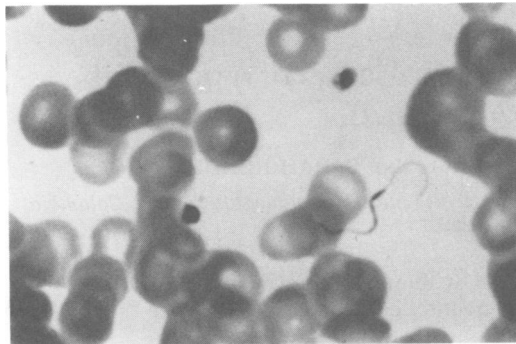
FIG. 1. *Exflagellated microgametocyte of P. vivax malaria (×1,000; Giemsa stain).*

remixed blood was 7.1 ± 0.1. In contrast, the pH of blood that was collected in tubes that contained ethylenediaminetetraacetic acid and then was poured into a petri dish to form a thin layer rose progressively over a 4-h period. From an initial pH 7.3 ± 0.1, the pH rose to 7.6 ± 0.1 at 1.5 h and to 7.7 ± 0.1 at 4 h after being placed in the open dish.

Discussion. In the life cycle of malaria parasites, exflagellation of the microgametocyte is observed in the mosquito gut cavity after ingestion of an infected blood meal. The sexual phase of the life cycle in the mosquito results in the formation of sporozoites which are introduced into another human during a subsequent blood meal. In humans, an asexual cycle in the liver and erythrocytes results. Development of the plasmodia occurs through trophozoite and merozoite forms to the gametocyte, but exflagellation is not observed. The microgametocyte and macrogametocyte, as part of the blood meal of the mosquito, continue transmission of the plasmodia. Between 1966 and 1979, there were only two reports of exflagellated microgametes in blood smears from patients with malaria (2, 6). In neither of the reports was exflagellation associated with *P. vivax* infection.

The primary factor that controls exflagellation in vitro is pH. With decreasing pCO_2 and rising pH, exflagellation is initiated. Although bicarbonate is required, Carter and Nijhout (1) have demonstrated that pH is the critical factor. Exflagellation has been observed in drops of blood exposed to air on a microscope slide with some *Plasmodium* species. It seemed that the most likely explanation for the phenomenon that we observed was a change in the milieu of the blood collection tube that facilitated exflagellation. Blood from the two patients with malaria had been collected into tubes that contained ethyl-

enediaminetetraacetic acid and subsequently sent to the laboratory. The blood smears were prepared within a few hours after the initial drawing. The several experiments with blood from patients who did not have malaria indicated that it is not easy to reach the requisite pH 7.7 under conditions that might be anticipated in a clinical laboratory. Only when blood was spread in a petri dish or the plasma was allowed to separate from the cells did the pH rise to levels that would produce exflagellation. The process took hours and was reversed when the plasma was remixed with blood. The possibility that conditions in the blood of the two patients produced a different effect in the collection tubes cannot be evaluated.

Exflagellation in the mosquito is more complex, however. Other factors in addition to pH participate in the initiation of exflagellation (5). It is unlikely, but not impossible, that some unidentified factor in these two patients produced exflagellation in vivo. Recently, Martin et al. (4) have shown that the phenomenon can be induced by phosphodiesterase inhibitors. They suggested that cyclic nucleotides may be involved, but the exact mechanism is not known. Interestingly, caffeine was the most potent agent investigated and the only one capable of activity at pH 7.4. The caffeine intake of our patients is not known.

The exflagellated microgamete is easily distinguished by size and morphology from other parasites, such as microfilaria or flagellated trypanosomes, and from spiral bacteria such as *Borelia* sp. Because exflagellation is associated with the development of the parasite in the mosquito stomach and not in humans, the appearance of the exflagellated microgamete in a blood smear presents a diagnostic challenge.

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