Plasmodium vivax Ookinetes in Human Peripheral Blood

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Received 8 April 1994/Returned for modification 31 May 1994/Accepted 12 July 1994

Microscopic examination of a peripheral blood smear revealed ookinetes of Plasmodium vivax. This unusual finding was probably due to an excessive delay between blood collection and smear preparation. Ookinete formation normally occurs in the mosquito gut. When seen in blood smears, it can cause confusion and misidentification of the parasite.

In the natural life cycle of the malarial parasites that infect humans, production of ookinetes occurs in the mosquito gut (1). When a mosquito ingests mature gametocytes in its blood meal, the gametocytes transform into gametes within 15 min (8). The macrogametocytes (female) transform into only one microgamete each, while exflagellation of a microgametocyte (male) produces several highly motile, very slender microgametes. A microgamete then penetrates a macrogamete, and the resulting zygote transforms into a fusiform, motile ookinete within 24 h (10).

The usual Plasmodium forms seen in human peripheral blood smears include the rings, trophozoites, and schizonts of the asexual, intraerythrocytic cycle and the macrogametocytes and microgametocytes of the sexual cycle. While exflagellation normally occurs in mosquitoes, it has been known for a long time that it can occur in vitro. In 1897, MacCallum (7) reported seeing gametocytes become flagellated forms while examining infected human blood under a microscope. Further, because exflagellating microgametocytes produce a visible disturbance of the erythrocytes immediately surrounding them (8), placing a drop of diluted, anticoagulated blood on a microscope slide and observing it for exflagellating microgametocytes is now the method used to judge the maturity of gametocytes. Thus, although we know that exflagellation occurs in vitro, the presence of microgametes on stained peripheral blood smears has rarely been reported (4, 11). Presented here is a case of Plasmodium vivax malaria in which exflagellating microgametocytes and ookinetes were found on a peripheral blood smear.

In April 1991, an 8-year-old girl from India was seen in a doctor's office. Since her symptoms were suggestive of malaria, blood was drawn into a collection tube containing EDTA. The blood remained in the tube from late afternoon until late that night, when a smear was prepared at a local laboratory. The smear was stained with Giemsa stain, and the parasites were identified at the laboratory as P. falciparum on the basis of the presence of what appeared to be crescent-shaped gametocytes. The smear was then forwarded to the Microbial Diseases Laboratory for species confirmation. Examination of the smear revealed typical intraerythrocytic asexual stages of P. vivax in enlarged erythrocytes with Schuffner's stippling, as well as exflagellating microgametocytes and microgametes (Fig. 1A). There were also forms that appeared to be either macrogametocytes or zygotes (2).

The fusiform cells were recognized as not being P. falcipa-
FIG. 1. Giemsa stain of a patient's peripheral blood showing the development of *P. vivax* ookinetes (magnification, ×1,000). Panels: A, exflagellating microgametocyte; B, very early retort form; C and D, progressively older retort forms; E, ookinete with vacuole (solid arrow) anterior to the nucleus; F, mature ookinete with vacuole (solid arrow) posterior to the nucleus. The apical pigment is visible (open arrow). Bar, 4 µm.
laboratory as possible mixed *Plasmodium* and *Borrelia* infections. It appears that microgametes can be mistaken for spirochetes by microscopists unfamiliar with their morphology.

The importance of correct collection of blood specimens for malaria diagnosis cannot be overemphasized. In standard procedures (5, 6), the preferred method is to make both thin and thick smears directly from blood collected by finger stick. If this is not practical, good smears can be made from blood drawn into EDTA as long as they are made soon after the blood is collected, preferably within 30 min but certainly within 1 h.

As can be seen from this case, ookinetes can form under conditions that may be encountered in clinical laboratories. Microscopists need to be aware of the possibility of finding these unusual forms of malaria parasites in peripheral blood smears.

REFERENCES