

Brief Report

**PLASMODIUM MALARIAE INFECTION IN
AN ASYMPTOMATIC 74-YEAR-OLD
GREEK WOMAN WITH SPLENOMEGALY**

JOSEPH M. VINETZ, M.D., JUN LI, M.D., PH.D.,
THOMAS F. McCUTCHAN, PH.D.,
AND DAVID C. KASLOW, M.D.

MALARIA most commonly presents as an acute systemic, febrile illness but may manifest more indolently as chronic anemia, glomerulonephritis, or tropical splenomegaly syndrome (or hyperreactive malarial splenomegaly) due to *Plasmodium falciparum*, *P. vivax*, or *P. malariae*.¹ Although all the major malaria parasites of humans cause acute illness that may be accompanied by splenomegaly, *P. malariae* is the only one recognized to cause asymptomatic infections that can last decades.¹ Asymptomatic *P. malariae* infections are typically associated with very low levels of parasitemia and normal physical examinations,^{2,3} and they generally come to medical attention when malaria is transmitted by transfusion^{2,3} or a recrudescence is induced by splenectomy performed for reasons unrelated to malaria.⁴

We report a case of malaria due to *P. malariae* in a 74-year-old woman from Greece whose illness was reactivated after decades of latency. All manifestations were reversed with a three-day course of chloroquine. Although thick and thin peripheral-blood smears were repeatedly negative, a nucleic acid-based assay detected the infecting species of malaria parasite. Since malaria was eradicated from Greece by about 1950, the patient's infection most likely lasted more than 40 years, possibly as long as 70. Symptoms of malaria began after asymptomatic splenomegaly was mistaken for lymphoma and she was treated with methotrexate. The fevers were in a quartan pattern (recurring every 72 hours). As Hippocrates wrote, "The least dangerous of all [fevers], and the mildest and most protracted, is the quartan."⁵

From the Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. (J.M.V., J.L., T.F.M., D.C.K.), and the Division of Infectious Diseases, Department of Medicine, Johns Hopkins School of Medicine, Baltimore (J.M.V.). Address reprint requests to Dr. Vinetz at the Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, 9000 Rockville Pike, Bldg. 4, Rm. 126, Bethesda, MD 20892.

©1998, Massachusetts Medical Society.

CASE REPORT

In 1994, splenomegaly was documented in a 72-year-old woman from the Greek island of Karpathos during a routine examination (her first in many years). The woman felt healthy, had no constitutional symptoms, denied ever having malaria, and had never traveled outside of Greece. At a hospital in Athens in September 1995, ultrasonography and computed tomography of the abdomen showed an enlarged spleen but no other abnormalities; the spleen measured 21 cm. The patient was given a diagnosis of lymphoma and advised to undergo splenectomy, which she declined. In October 1995, she was treated with oral methotrexate (10 mg per day for 10 days). After seven days, rigors, headache, and fever (temperature of up to 39°C) developed that recurred in a quartan pattern for five cycles and that resolved after the methotrexate was stopped. Evaluation, including several blood films, provided no explanation for these symptoms.

When the woman was evaluated for splenomegaly in our clinic in February 1996, she denied all symptoms of illness. The physical examination was normal, except for a nontender, firm spleen extending 14 cm below the costal margin. Relevant laboratory data are shown in Table 1. The results of hemoglobin electrophoresis were normal, and glucose-6-phosphate 1-dehydrogenase levels were normal. Renal function and liver function, assessed by serum chemical analyses, were normal, as were the results of urinalysis. Serum IgG levels were elevated (Table 1). Serum levels of IgM were low (35 mg per deciliter). Five thick and five thin blood films obtained over a two-week period were negative for plasmodium species (more than 500 fields per slide were examined, corresponding to a finding of fewer than 2 parasites per microliter of blood).

The patient was given a conventional three-day course of chloroquine. One month later the spleen was no longer palpable; at six months, the size of the spleen was normal (13 cm) on ultrasonography. The patient's older sister reported that the patient had had malaria at approximately three years of age (around 1925) that was never treated and that spontaneously resolved.

In May 1997, one year after chloroquine treatment, we reevaluated the patient. She continued to feel clinically well and had gained 10 kg (22 lb). No abnormalities were found on physical examination. The spleen was no longer palpable, and ultrasonography confirmed that the spleen was of normal size (13 cm in the longest axis). Indirect immunofluorescence showed that antibodies against *P. malariae* had declined by a factor of 4 (Table 2). Serum IgG and hemoglobin levels had returned to normal (Table 1).

METHODS

The study was approved by the Johns Hopkins Medical Institutions Joint Committee on Clinical Investigation and the National Institutes of Health Human Subjects Committee. The patient gave written informed consent.

Antibodies against all four malaria parasites of humans were measured by an immunofluorescence assay at the Centers for Disease Control and Prevention, Atlanta.^{6,7} The presence of *P. malariae* was evaluated by determining the species-specific sequence of an 18S ribosomal RNA (rRNA) in blood samples from the patient. This test can detect one plasmodium cell in 500 μ l of whole blood.⁸ Parasite rRNA was reverse transcribed into single-stranded complementary DNA that was then amplified with the polymerase chain reaction (PCR), as previously described.⁸ The design of the PCR primers was based on highly conserved sequences in the 18S rRNA gene from the genus plasmodium: 5' primer 841 (5'GAACGAGATCTTAACCTGC3'); 3' primer 844 (5'TAITG-ATAAAGATTACCTA3'). The resulting products were separated by electrophoresis and Southern blot hybridization with a ³²P-labeled oligonucleotide probe specific for *P. malariae* rRNA (5'TTTCACCTTAAGAATATAGTGTATT3'). Amplified DNA fragments of rRNA were cloned as described previously.⁸ DNA sequencing was done manually by a modification of the dideoxynucleotide termination method of Sanger (Sequenase 2.0,

TABLE 1. LABORATORY DATA BEFORE AND AFTER CHLOROQUINE TREATMENT.

VARIABLE	BEFORE CHLOROQUINE	ONE YEAR AFTER CHLOROQUINE
Hemoglobin (g/dl)	12	14
Mean corpuscular volume (fl)	85	86
Platelets (per mm ³)	191,000	230,000
Serum IgG (mg/dl)	2,120	1,240

TABLE 2. RESULTS OF AN INDIRECT IMMUNOFLUORESCENCE ASSAY FOR THE PRESENCE OF ANTIPLASMODIUM ANTIBODIES IN THE PATIENT'S BLOOD.*

PARASITE	BEFORE CHLOROQUINE	ONE YEAR AFTER CHLOROQUINE
	titer	
<i>P. vivax</i>	1:4096	1:256
<i>P. falciparum</i>	1:65,536	1:16,384
<i>P. malariae</i>	1:262,144	1:65,534
<i>P. ovale</i>	1:1024	1:256

*A titer greater than 1:64 is considered positive.

U.S. Biochemical, Cleveland). The sequence of the *P. malariae* rRNA fragment reported here has GenBank accession number AF014942.

RESULTS

Five specimens of whole blood, collected in citrate and simultaneously used for blood smears, were obtained over a two-week period and stored at 4°C. Thin and thick smears of all specimens were negative. Reverse-transcription (RT) PCR with genus-specific primers for plasmodium 18S asexual-stage rRNA was performed on total RNA extracted from 50 µl of whole blood. A Southern blot of the amplified DNA was hybridized with an internal probe specific for the *P. malariae* 18S rRNA gene (Fig. 1). Two of five specimens showed the presence of *P. malariae* rRNA, suggesting that the degree of parasitemia was most likely near the limit of detection. Sequence analysis of PCR-amplified DNA fragments from the patient's specimens further confirmed the presence of an infection with *P. malariae* (Fig. 2); the sequence amplified from the patient differed slightly from another *P. malariae* sequence determined in our laboratory (GenBank accession number U78741).

Two specimens obtained one month after chloro-

quine treatment were negative on the basis of both ethidium-stained agarose-gel fractionation and Southern blot hybridization of RT-PCR products. An indirect immunofluorescence assay showed high titers of antiplasmodium antibodies, highest against *P. malariae*, which had declined to one fourth of that level one year after chloroquine treatment (Table 2).

DISCUSSION

This case demonstrates that *P. malariae* can cause prolonged asymptomatic infection that can reactivate decades after the initial infection and manifest as an indolent illness associated with insidious weight loss, splenomegaly, anemia, and hypergammaglobulinemia. On the basis of documentation provided by the World Health Organization (Dzenowagis J: personal communication) and others,^{9,10} the transmission of malaria was essentially eradicated in Greece, including Peloponnesus and Karpathos, by the early 1950s. Thus, the patient had most likely had this infection for more than 40 years. The clinical history suggests that her infection could have lasted about 70 years.

Although long-term asymptomatic infection with *P. malariae* is a well-recognized clinical entity, this case has a number of unusual features. First, the infection was suspected on the basis of the finding of splenomegaly; patients with chronic *P. malariae* typically have normal physical examinations.² In contrast, acute malaria due to any of the four malaria parasites of humans is often associated with splenomegaly, which is also common in regions in which malaria is endemic among residents, with chronic or recurrent malaria due to *P. falciparum* or *P. vivax*. Second, despite the fact that more than 10 blood smears were negative over a period of more than 15 months, we were able to detect the infecting species of malaria parasite using an RT-PCR assay that provides a species-specific diagnosis. Third, serologic analysis was useful in confirming the clinical diagnosis of malaria. Typically, the indirect immunofluorescence assay is helpful for excluding the diagnosis of malaria; a positive titer does not confirm current infection, but rather that infection has occurred at some time in the past.¹¹

Finally, that the patient's methotrexate treatment induced a malarial quartan fever seems certain: she had never had acute symptoms of fever or malaria previously, the symptoms disappeared soon after the methotrexate was stopped, and the pattern of fever was characteristic. Curiously, methotrexate is lethal to malaria parasites¹²; the blood levels of methotrexate must have been sublethal to the parasite in our patient but presumably sufficient to interfere with cell-mediated immunity, as occurs, for example, in rheumatoid arthritis and systemic lupus erythematosus.¹³ Because neither antibody titers nor antibody

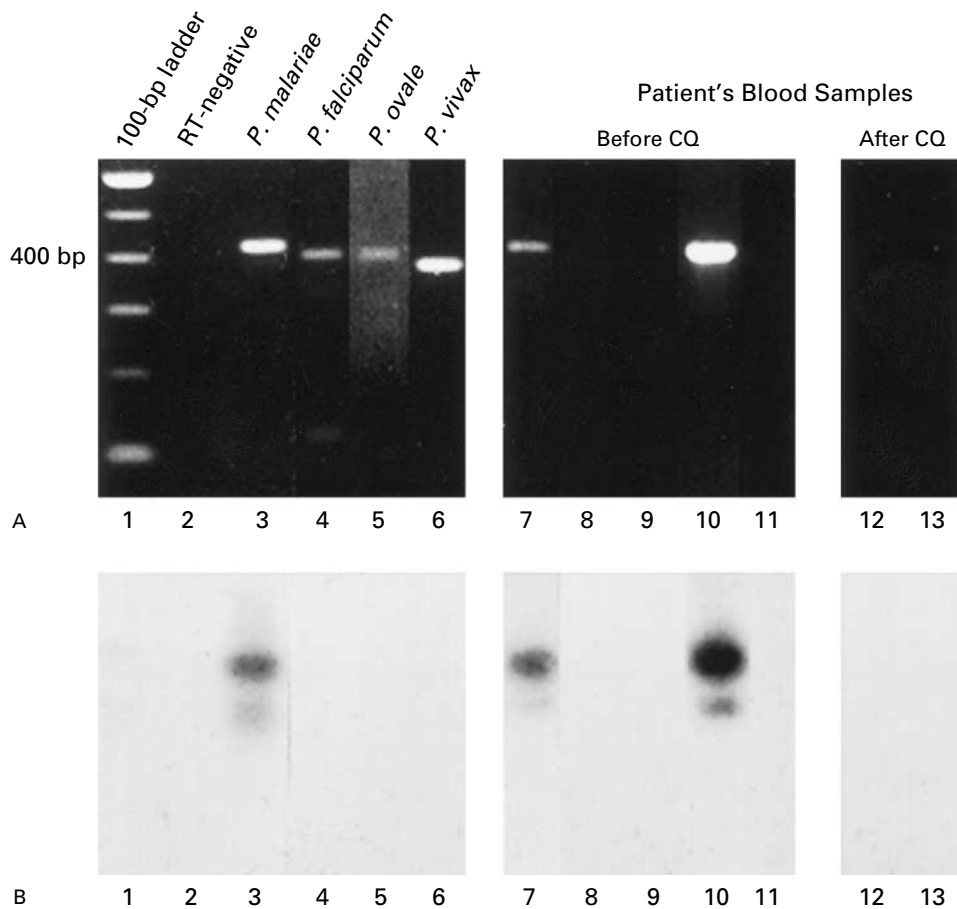


Figure 1. Identification of 18S *Plasmodium* Ribosomal RNA from Whole-Blood Specimens from the Patient by Staining with Ethidium Bromide (Panel A) and Southern Blot Hybridization (Panel B).

Total RNA was extracted from control laboratory specimens of malaria parasites (lanes 3, 4, 5, and 6) and five serial blood specimens obtained from the patient before chloroquine (CQ) treatment (lanes 7, 8, 9, 10, and 11) and one year after treatment (lanes 12 and 13). The RNA was subjected to reverse transcriptase (RT) PCR as described in the Methods section. In Panel A, ethidium bromide staining of agarose gels shows the presence of *P. malariae* ribosomal RNA in two specimens from the patient. In Panel B, Southern blot hybridization with a ^{32}P -labeled *P. malariae*-specific oligonucleotide probe again shows the presence of *P. malariae* ribosomal RNA in two specimens from the patient. Lane 2 shows a negative control for identifying genomic-DNA contamination.

specificities would be expected to change within one week after immunosuppression induced by treatment with methotrexate, the rapid appearance of malaria symptoms after the administration of methotrexate suggests that cellular immunity contributed to the control of *P. malariae* infection in this patient. *P. inui*, a quartan-malaria parasite of monkeys thought to be analogous to *P. malariae*, has been reported to recur as a consequence of T-cell immunosuppression due to infection with the simian immunodeficiency virus.¹⁴ In contrast, neither the severity nor the frequency of illness due to *P. falciparum*, the lethal malaria parasite of humans, seems to be exacerbated by T-cell immunosuppression from concomitant infec-

tion with the human immunodeficiency virus,¹⁵⁻¹⁷ despite data from murine models of malaria suggesting that T cells are central to both induction and effector mechanisms of naturally acquired protection.^{18,19}

The differential diagnosis was difficult in this case, especially given the paucity of systemic manifestations. In the tropics, a finding of splenomegaly has a broad range of diagnostic possibilities.^{20,21} Consideration in the present case was given to whether tropical splenomegaly syndrome (also known as hyperreactive malarial splenomegaly)²² was present. This syndrome is characterized by fever, anemia, weight loss, abdominal discomfort, and lassitude. Laborato-

Supported by a Physician Postdoctoral Fellow Award from the Howard Hughes Medical Institute (to Dr. Vinetz).

Presented in part at the 45th Annual Meeting of the American Society for Tropical Medicine and Hygiene, Baltimore, December 1-5, 1996, and at the Infectious Disease Society of America Annual Meeting, San Francisco, September 7-11, 1997.

We are indebted to Maria Nikolaidis for her help in gathering evidence for this report; to William Collins, Ph.D., and Marianna Wilson, M.S., of the Parasitology Branch, Centers for Disease Control and Prevention, Atlanta, for performing monkey and serologic studies and for helpful discussions; to J. Dzenowagis, Ph.D., World Health Organization, Geneva, for obtaining archival information about malaria transmission in Greece; to Drs. Franklin Neva, Louis Miller, Theodore Nash, Thomas Nutman, David Fidock, Mary Marovich, and Philip Cooper for helpful discussions and critique of the manuscript; to Thomas Spahr of the Johns Hopkins Hospital Clinical Microbiology Laboratory, for preparing blood smears and handling the specimens; to the Malaria Internet Group for obtaining information about the history of malaria transmission in Greece; and to Nancy Shulman for helping to prepare the manuscript.

REFERENCES

1. Krogstad DJ. Plasmodium species (malaria). In Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas and Bennett's principles and practice of infectious disease. 4th ed. Vol. 2. New York: Churchill Livingstone, 1995:2415-27.
2. Guerrero IC, Weniger BG, Schultz MG. Transfusion malaria in the United States, 1972-1981. *Ann Intern Med* 1983;99:221-6.
3. Bruce-Chwatt LJ. Blood transfusion and tropical disease. *Trop Dis Bull* 1972;69:825-62.
4. Tsuchida H, Yamaguchi K, Yamamoto S, Ebisawa I. Quartan malaria following splenectomy 36 years after infection. *Am J Trop Med Hyg* 1982; 31:163-5.
5. Hippocrates. The epidemics. Book 1. In: The genuine works of Hippocrates. Adams F, trans. New York: William Wood, 1886:307.
6. Sulzer AJ, Wilson M. The fluorescent antibody test for malaria. *CRC Crit Rev Clin Lab Sci* 1971;2:601-19.
7. Sulzer AJ, Wilson M, Hall EC. Indirect fluorescent-antibody tests for parasitic diseases. V. An evaluation of a thick-smear antigen in the IFA test for malaria antibodies. *Am J Trop Med Hyg* 1969;18:199-205.
8. Li J, Wirtz RA, McConkey GA, et al. Plasmodium: genus-conserved primers for species identification and quantitation. *Exp Parasitol* 1995;81: 182-90.
9. Livadas GA. Malaria eradication in Greece. *Rivista di Malariologia* 1958;37:173-91.
10. Belios GD. Recent course and current pattern of malaria in relation with its control in Greece. *Rivista di Malariologia* 1955;34:1-24.
11. Wilson M, Sulzer AJ, Runcik K. Malaria-antibody patterns as determined by the IFA test in U.S. servicemen after chemotherapy. *Am J Trop Med Hyg* 1970;19:401-4.
12. Walter RD, Bergmann B, Kansy M, Wiese M, Seydel JK. Pyrimethamin-resistant *Plasmodium falciparum* lack cross-resistance to methotrexate and 2,4-diamino-5-(substituted benzyl) pyrimidines. *Parasitol Res* 1991;77:346-50.
13. Fox DA, McCune WJ. Immunologic and clinical effects of cytotoxic drugs used in the treatment of rheumatoid arthritis and systemic lupus erythematosus. *Concepts Immunopathol* 1989;7:20-78.
14. Hirsch VM, Goldstein S, Hynes NA, et al. Prolonged clinical latency and survival of macaques given a whole inactivated simian immunodeficiency virus vaccine. *J Infect Dis* 1994;170:51-9.
15. Simooya OO, Mwendapole RM, Sikateyo BM. Severe falciparum malaria and the acquired immunodeficiency syndrome (AIDS) in Zambia. *Ann Trop Med Parasitol* 1991;85:269-70.
16. Greenberg AE, Nsa W, Ryder RW, et al. *Plasmodium falciparum* malaria and perinatally acquired human immunodeficiency virus type 1 infection in Kinshasa, Zaire. *N Engl J Med* 1991;325:105-9.
17. Muller O, Moser R. The clinical and parasitological presentation of *Plasmodium falciparum* malaria in Uganda is unaffected by HIV-1 infection. *Trans R Soc Trop Med Hyg* 1990;84:336-8.
18. Cavacini LL, Parke LA, Weidanz WP. Resolution of acute malarial infections by T-cell-dependent non-antibody-mediated mechanisms of immunity. *Infect Immun* 1990;58:2946-50.
19. Kumar S, Good MF, Dontfrad F, Vinetz JM, Miller LH. Interdependence of CD4⁺ T cells and malarial spleen in immunity to *Plasmodium vinckei vinckei*: relevance to vaccine development. *J Immunol* 1989;143: 2017-23.
20. Schaefer K-U, Khan B, Gachihi GS, et al. Splenomegaly in Baringo District, Kenya, an area endemic for visceral leishmaniasis and malaria. *Trop Geogr Med* 1995;47:111-4.
21. Cook GC, ed. Manson's tropical diseases. 20th ed. London: W.B. Saunders, 1996:23.
22. Fakunle YM. Tropical splenomegaly. 1. Tropical Africa. *Clin Haematol* 1981;10:963-75.
23. Bottius E, Guanzirolli A, Trape J-F, Rogier C, Konate L, Druilhe P. Malaria: even more chronic in nature than previously thought; evidence for subpatent parasitaemia detectable by the polymerase chain reaction. *Trans R Soc Trop Med Hyg* 1996;90:15-9.
24. Tikasingh E, Edwards C, Hamilton PJS, Commissiong LM, Draper CC. A malaria outbreak due to *Plasmodium malariae* on the island of Grenada. *Am J Trop Med Hyg* 1980;29:715-9.