Continuation of Chloroquine-Susceptible *Plasmodium falciparum* Parasitemia in Volunteers Receiving Chloroquine Therapy

JAMES R. MURPHY,†* DAVID F. CLYDE, DEIRDRE A. HERRINGTON, SHAHIDA BAQAR,
JONATHAN R. DAVIS, KATHLEEN PALMER, AND JOSEPH CORTESE

Center for Vaccine Development, Department of Medicine, School of Medicine,
University of Maryland, Baltimore, Maryland 21201

Received 15 September 1989/Accepted 19 January 1990

Volunteers infected with a chloroquine-susceptible line of *Plasmodium falciparum* were administered standard oral chloroquine therapy at the first detection of parasites in the blood. Parasitemia progressed in the face of therapy for up to 5 days and to levels up to 100-fold greater than those at the initiation of treatment. Thereafter, infections cleared without a requirement for additional chemotherapy. This course of infection and response to treatment has not been previously reported and may have been detected because volunteers were exposed to an unusually large number of sporozoites. The observations are consistent with the hypothesis that prolonged parasitemia resulted from the continued release of merozoites from liver.

Oral administration of chloroquine to individuals with chloroquine-susceptible *Plasmodium falciparum* infection has been shown to result in the rapid arrest (within hours) and clearance (within days) of asexual erythrocytic parasites (16). In contrast to this expected pattern of response, levels of parasitemia in some volunteer participants in a recent study of induced *P. falciparum* infection showed an increase for up to 5 days after the initiation of chloroquine treatment. For some individuals, the parasitemia 2 to 4 days after the start of treatment was 10 to 100 times greater than was the parasitemia at the initiation of treatment. Parasites causing the persisting parasitemias were chloroquine susceptible both in vivo and in vitro; thus, the continued development of infection could not be attributed to resistance to the drug. In this report, we present the course of these infections and offer explanations for continued chloroquine-susceptible *P. falciparum* parasitemia in the face of chloroquine treatment.

Data were obtained from 16 adult (median age, 24 years; range, 21 to 33 years) male participants in three separate studies in which *P. falciparum* NF-54 infections were induced through bites of about five infected mosquitoes per volunteer. Informed consent was obtained from all of the participants in these studies. The guidelines for human experimentation of the U.S. Department of Health and Human Services were followed in the conduct of the clinical research, and approval was obtained from the University of Maryland Human Volunteer Research Committee. These studies were part of clinical trials of a *P. falciparum* sporozoite vaccine (8, 9; D. A. Herrington et al., unpublished data). Four individuals had received vaccination against *P. falciparum* sporozoites [synthetic peptide (NANP), conjugated to a tetanus toxoid carrier and adsorbed to aluminum hydroxide]; twelve individuals had no history of contact with malaria or malaria vaccines. Details of the vaccine, the clinical trials, and the challenge model are presented elsewhere (8, 9; D. A. Herrington et al., unpublished).

Thick blood smears were prepared from all volunteers twice daily, at about 7 a.m. and 7 p.m. beginning on day 5 after mosquito bites and continuing until after parasitemia was cleared. Following staining with Giemsa, 500 leukocytes were counted and the number of parasites found during the leukocyte count was recorded. The number of parasites per milliliter was estimated by adjusting the number per 500 leukocytes for the number of leukocytes per milliliter (determined by an electronic cell counter).

Treatment with chloroquine phosphate was instituted in all volunteers as soon as parasitemia was confirmed by microscopic examination of a thick blood film. A total of 600 mg of chloroquine base was administered orally followed by 300 mg 6, 24, and 48 h after the first dose.

Panels 9 through 16 of Fig. 1 present the courses of *P. falciparum* infections in eight volunteers who were challenged on 7 January 1988 (study 3) by bites of a single group of infected mosquitoes. Infection in vaccinees was detected, on average, a few days later than infection in nonimmunized controls; however, once patenty was established, the pattern of infection was similar for both groups. Parasitemia continued for an average of 4.5 days after the first dose of chloroquine; all volunteers had at least one rise in parasitemia after the initiation of chloroquine therapy. At times, posttherapy parasitemia was 100 times as high as it was at the initiation of therapy. Among individuals, the interval from the first to last detection of parasites ranged from 2 to 5 days; individual parasitemias varied from the lower limit of detection (about 6,000 to 10,000 parasites per ml) to about 1,500,000 parasites per ml. Particularly impressive were the marked changes in the level of parasitemia within short intervals. For example, the infection presented in panel 15 at days 9 and 10 was about 10,000 parasites per ml, yet at day 11, parasitemia was 1,500,000 parasites per ml. Thick smears prepared after day 14 were negative for *P. falciparum*. The single course of chloroquine initiated at the indicated times was sufficient to cure infection in all volunteers.

Because of the preceding result, we reviewed the evolution of parasitemias in volunteers in two previous studies that used the same in vitro-cultured strain of *P. falciparum*. Panels 1 through 8 of Fig. 1 present parasitemias for infections of nonimmunized volunteers; data are from two separate infections, each initiated with a separate group of mosquitoes (study 1, initiated 31 January 1986, is presented in panels 1 through 4; study 2, initiated 20 April 1987, is presented in panels 5 through 8).

* Corresponding author.
† Present address: 632 Overbrook Road, Baltimore, MD 21212.
In study 1, there were two peaks of parasitemia in one volunteer (at days 9.5 and 11.5; Fig. 1, panel 2) and a single peak at day 11.5 for the remaining three individuals. Parasites were not found at intervals beyond 12.5 days. Infections became patent in study 2 between 7.5 and 10.5 days after mosquito bites. Three of the four volunteers had parasitemias which persisted for some interval after the initiation of treatment. Two individuals showed posttreatment parasitemias which were somewhat higher than those observed at the initiation of therapy. All thick smears prepared after day 12 were negative for *P. falciparum*.

Continued parasitemia after chloroquine treatment of chloroquine-susceptible *P. falciparum* infection was a surprising finding; we have not found other reports of this course of infection. To explain this observation, we surmise that our use of procedures different from those employed previously may have enabled our detection of the reported pattern of infection, i.e., thick smears prepared at 12-h intervals from 5 through at least 13 days after infective mosquito bites and the induction of infections with a laboratory-adapted cultured line of *P. falciparum* which allowed an unnaturally large number of sporozoites to be injected (5).

Changes in the laboratory strain of *P. falciparum* NF-54 or the procedures used appear to have resulted in a progressive increase in the number of sporozoites inoculated during the 2-year interval over which these studies were conducted (Table 1). Recently, an analysis of sporozoite equivalents for mosquitoes from the group used for challenge in study 3 was published (5); these mosquitoes had an average of 220,000 sporozoite equivalents per insect. This is unlike the case in which field-caught specimens were tested; in the latter case, there was an average of about 6,000 sporozoite equivalents per mosquito (for a review, see reference 5). This approximately 40-fold difference in whole mosquito sporozoite number seems to have been reflected in the number of sporozoites delivered as challenge. Our composite result is
consistent with the view that those mosquitoes used for study 3 transmitted an unnaturally large number of sporozoites to volunteers. We believe that the large number of sporozoites delivered in study 3 contributed to our capacity to track recurrent waves of parasitemia. If this view is correct, the patterns of parasitemia reported here would not be expected to be observed for malaria naturally acquired in the field.

The proximal source of the parasites which comprised the late peaks in parasitemia is unknown. These could have been progeny of asexual erythrocytic stages which had reached a chloroquine-insusceptible form (i.e., schizonts [18]) prior to the initiation of chloroquine therapy or which were sequestered in chloroquine-protected sites. This scenario is unlikely because the timing and magnitude of late peaks are difficult to reconcile with the known duration of the asexual erythrocytic cycle and the number of merozoites produced per schizont (1, 15).

An alternative explanation is that continued parasitemia is attributable to continued merozoite release from the liver. It is known that liver infection is refractory to chloroquine therapy (2). Further, because chloroquine does not inhibit merozoite invasion into erythrocytes (18), parasites released from the liver would be expected to establish parasitemia in the face of chloroquine and thus, if released in sufficient number, be detected in thick smears. In the presence of chloroquine, parasites would not be expected to complete erythrocytic schizogony (13, 18). The lack of fever (i.e., temperature >37.8°C; Fig. 1) in association with late peaks of parasitemia in study 3 is consistent with a failure of schizogony, since fever is associated with the rupture of schizonts (3).

The observed pattern of parasitemia suggests the discontinuous release of merozoites from the liver with peaks of release occurring at about infection days 7, 9, and 11 to 12. Because it is established that human P. falciparum infection can comprise genetically different parasites (14) and because the P. falciparum used in these studies, NF-54, was not cloned, it is possible that recurrent waves of parasitemia reflect divergent exoerythrocytic maturation rates of genetically different lines.

It is informative to view the results from the perspective of the reporting scheme for chloroquine resistance of the World Health Organization (WHO) (17). Specifically, in reference to the WHO Standard Test, which includes a 7-day postinfection of therapy observation period, our demonstration of markedly increased parasitemia shortly after the initiation of therapy fits the pattern for RIII chloroquine resistance (the highest degree of resistance in the scheme). On the other hand, the demonstration that parasitemia was reduced by the single course of therapy to below detectable limits by 7 days after the initiation of treatment and that parasitemia did not reappear after day 7 fits the S classification (the greatest degree of susceptibility to chloroquine). Thus, the pattern of response observed seems an exception to the long-standing WHO scheme. We conclude that the explanation offered above is the most likely reason for the unexpected result. A possible alternative explanation would be that chloroquine treatment is more effective when applied against parasitemias of greater density (and of longer duration) than when initiated at very low parasitemia (WHO tests are normally performed when parasitemia is between 500,000 and 1,000,000 parasites per ml). We know of no evidence to support this possibility.

In future tests of vaccines against erythrocytic stages of P. falciparum, infections might be initiated either by intravenous injection of erythrocytes containing parasites or by injection of sporozoites by mosquito bite. The findings summarized in this report suggest that these methods might present very different challenges to host defenses. Injection of erythrocytic forms would represent a point source of parasites. In contrast, following sporozoite injection, parasites might be released into blood in waves over an interval of days and in numbers much larger than the number of parasites which would likely be contained in an injection of infected erythrocytes. The possibility that the first waves of parasites might modulate the capacity of host defenses to respond to subsequent releases of merozoites must be considered (11).

We thank the volunteers and the clinical staff of the Center for Vaccine Development for making these studies possible. Financial support was received from the U.S. Agency for International Development, the American Institute of Biological Sciences (contract 8074), and the National Institute of Allergy and Infectious Diseases (contract N01-AI62533).

LITERATURE CITED


