In *Plasmodium falciparum* malaria, there is evidence for the chronicity of the disease (2, 5, 7, 9). It is thought that the chronic status of *P. falciparum* infection facilitates the acquisition of protective immunity against superinfections of this parasite; however, this acquired immunity is incomplete (2, 4, 5, 9). Individuals with such immunity are able to control malaria parasites at low parasitemia levels, but they cannot eradicate them (2, 5, 9). The term premunition has been used by malarialogists to refer to this immune phenomenon (2, 4, 5, 9, 10). Although there have been reports of malarial premunition in both human (5, 7, 9, 10) and animal models (1, 4), there have been no investigations systematically describing the kinetics of malaria infection under premunition.

Besides immunological influences, malaria infection is controlled by antimalarial treatments. Inadequate antimalarial treatments may prolong the presence of malaria parasites in the host (12).

In this study, we evaluated the influence of antimalarial treatment on the acquisition of immunity against parasites and investigated the course of malaria infection in mice with premunition.

Naive female BALB/c mice (purchased from the Japan Charles River Company and bred in the Animal Research Center for Tropical Infectious Diseases, Institute of Tropical Medicine, Nagasaki University) were intraperitoneally infected with $10^6$ parasitized red blood cells (pRBCs) of virulent *Plasmodium berghei* NK65 parasites (11). The infected mice received curative treatments in order to treat primary infection and chronic status. The method of Eling and Jerusalem (3, 4) was modified and applied in this study such that curative treatments were provided to the infected mice in the form of daily drinking water containing either 15 or 30 mg of sulfadiazine (Sigma Chemical Co., St. Louis, Mo.) per liter of water (hereafter called SF15 and SF30 therapy, respectively). These curative treatments started on day 3 (when parasitemia was around 1 to 2%) and were discontinued on day 30 of the primary infection. In addition, a radical treatment was applied in another mouse group receiving SF30 therapy: SF30 therapy was simultaneously combined with chloroquine (CQ) treatment. CQ (10 mg per kg of body weight per day; Sigma Chemical Co.) was intraperitoneally injected on four consecutive days every week. This regimen of CQ injection started on the same day as SF30 therapy (day 3) and was repeated four times throughout the course of SF30 therapy. Parasites were eradicated by the CQ treatment in all mice from day 31 to day 35 of the primary infection, so that parasitemia was negative at the time of reinfection. On day 60 of primary infection, the mice were intraperitoneally reinoculated with $10^5$ pRBCs of *P. berghei* NK65. Parasitemia was examined by microscopic detection on Giemsa-stained thin blood smears. Data processing and statistical analysis were conducted by using SPSS for Windows software, version 10.0. A *P* value of $\leq 0.05$ was considered significant.

As a result, the parasites were microscopically undetectable throughout the primary infection in all mice (5 of 5) in the radical treatment group, whereas parasite recrudescence occurred in all mice (59 of 59) in the curative treatment groups. These showed that the primary infection was well suppressed in mice in the radical treatment group but remained chronic in mice in the curative treatment groups.

During reinfection, 100% (5 of 5) of the mice that received the radical treatment during the primary infection died, whereas only 11.9% (7 of 59) of the mice that received the curative treatments died ($P < 0.001$). In the SF15 curative treatment group, 5.7% (2 of 35) of the mice died, and in the SF30 curative treatment group, 20.8% (5 of 24) of the mice died ($P = 0.109$). This suggests that most of the mice in the curative treatment groups became immunized and that only parasite persistence during primary infection could induce a considerable level of protective immunity in the infected mice.

With regard to the kinetics of parasitemia during reinfection in immunized mice in the curative treatment groups, parasitemia was suppressed to undetectable levels during the first week, but afterwards it reappeared at a low grade (peaks of parasitemia percentages around 1%) for a few days and subsequently became undetectable again (Fig. 1). That fluctuation of parasitemia was repeated three to four times throughout the 3-month reinfection (data not shown). This pattern of parasitemia was typical in the mice in the SF15 therapeutic group. This fluctuation of parasitemia (Fig. 1) showed that the immu-
Effective immunity against parasites than did the higher dose (SF30 therapy and radical treatment). As mentioned earlier, protective immunity may not be acquired if antimalarial treatment eradicates parasites from the start of infection. These findings may be in agreement with efforts to develop vaccines that reduce the parasite burden or neutralize the pathogenic properties without eliminating the parasite, thereby maintaining the immunity by continuous natural boosting (6).

In contrast to the original method of Eling and Jerusalem (3, 4), SF15 therapy in our model with *P. berghei* NK65 parasites and female BALB/c mice may better promote the immunity of mice because the mortality of our immunized mice during reinfection (5.7%) was lower than that of mice immunized according to Eling’s method (15%) (4). The phenomenon of malarial premunition during reinfection in these immunized mice was typical and very reproducible. This model may be useful for investigations of the immune mechanisms underlying malarial premunition.

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