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Influence of Antimalarial Treatment on Acquisition of Immunity in *Plasmodium berghei* NK65 Malaria

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Antimalarial treatments during primary *Plasmodium berghei* NK65 infection in BALB/c mice influenced the acquisition of protective immunity against reinfection. Among subcurative treatments, lower doses better enable mice to acquire protective immunity than do higher doses. Eradication of parasites from the start of infection did not promote protective immunity.

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 malariologists 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 subcurative treatments in order to maintain primary infection and chronic status. The method of Eling and Jerusalem (3, 4) was modified and applied in this study such that subcurative 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 subcurative 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 reinfected 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 ≤ 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 subcurative 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 subcurative 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 subcurative treatments died ($P < 0.001$). In the SF15 subcurative treatment group, 5.7% (2 of 35) of the mice died, and in the SF30 subcurative treatment group, 20.8% (5 of 24) of the mice died ($P = 0.109$). This suggests that most of the mice in the subcurative 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 subcurative 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-

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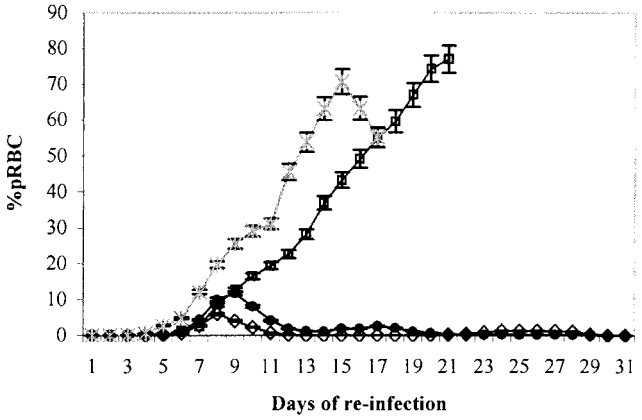

FIG. 1. Course of reinfection of virulent *P. berghei* NK65 parasites in mice that were previously infected with *P. berghei* NK65 parasites and treated with either subcurative treatment (SF15 or SF30 therapy) or radical treatment (SF30 therapy combined with CQ injection). ◇, data for mice in the SF15 therapeutic group; ●, data for mice in the SF30 therapeutic group; □, data for mice in the radical treatment group; ×, data for mice in the control group (naive mice infected with *P. berghei* NK65 and not treated with any antimalarial drugs). All data on the graph are mean values of parasitemia percentages. Error bars on the *y* axis represent 5% differences from the mean values. The curves for the SF15 and SF30 treatment groups indicate recrudescence and fluctuation of parasitemia during reinfection of the mice in the subcurative treatment groups. The curve for the radical treatment group indicates reinfection in mice of the radical treatment group that was not controlled.

nity acquired during the chronic primary infection was effective in suppressing parasite growth during reinfection but inadequate in completely eliminating the parasites. This may correspond to the characteristics of premunition, in which a very potent antiparasitic action can suppress a mass of parasites but allows parasites to persist at a low level (2). According to its definition (2, 8, 9) and observations (4, 5, 9, 10), premunition was established in the mouse model of this study.

Within the subcurative treatment groups, the mean clearance time of the initial parasitemia during the first 2 weeks of reinfection in immunized mice in the SF15 therapeutic group (8.67 ± 1.23 days [mean ± standard deviation]) was significantly shorter than that of mice in the SF30 therapeutic group (11.68 ± 2.98 days) (P, <0.001; 95% confidence interval, 1.46 to 4.57 days). The mean parasitemia peak (pRBC peak) during the first 2 weeks of reinfection of the immunized mice in the SF15 therapeutic group (4.92% ± 2.64%) was lower than that of the mice in the SF30 therapeutic group (14.58% ± 7.11%) (P, <0.001; 95% confidence interval, 6.01 to 13.31%), and the mortality of the immunized mice in the SF15 therapeutic group (5.7%) was lower than that of the mice in the SF30 therapeutic group (20.8%) (P = 0.109). This suggests that the lower therapeutic dose (SF15 therapy) enabled the host to acquire more-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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