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Recombinant Human Gamma Interferon Inhibits Simian Malaria†

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Prophylactic treatment with 0.1 mg of human gamma interferon per kg (body weight) per day completely suppressed experimental infection with *Plasmodium cynomolgi* B sporozoites in rhesus monkeys. Treatment with lower doses partially suppressed this infection. Prophylactic treatment with human gamma interferon, however, had no protective effect against trophozoite-induced infection, suggesting that the interferon effect was limited to the exoerythrocytic stage of parasitic development.

Treatment with interferon inducers or with crude interferon preparations containing alpha interferon and beta interferon significantly inhibited *Plasmodium berghei* malaria infection in mice (5–8, 18). In most of the studies, all carried out before 1970, the site of interferon action appeared to be on the pre- or exoerythrocytic phase of the parasite life cycle (5–8), although in one study (18) crude mouse serum interferon was reported to decrease the capacity of parasitized mouse erythrocytes to initiate lethal *P. berghei* infections in mice. In the other studies crude mouse serum interferon appeared to be an effective prophylaxis against sporozoite-induced *P. berghei* malaria (5–8). In a recent study by us, mice treated with purified mouse alpha and beta interferons were also protected against sporozoite-induced infections with *P. berghei* (R. K. Maheshwari, M. Hollingdale, and R. M. Friedman, unpublished results).

The cloning of human interferon genes has allowed the production of quantities of purified human interferon subtypes sufficient to study their effect on malaria infection in primates (4). We have tested the prophylactic effect of *E. coli*-derived recombinant human gamma interferon (rHuIFN-γ) against sporozoite- or trophozoite-induced *Plasmodium cynomolgi* B malaria in rhesus monkeys (16). The course of the infection of rhesus monkeys with *P. cynomolgi* B closely resembles that of *Plasmodium vivax* in humans, especially with respect to recurrent relapses after chloroquine therapy (17). In this study we present the first evidence that in primates rHuIFN-γ can inhibit infection caused by a nonviral pathogen. Our results indicated that rHuIFN-γ has a potent inhibitory effect on sporozoite-induced malaria, but no effect on trophozoite-induced infection.

MATERIALS AND METHODS

Monkeys. Rhesus monkeys (*Macaca mulatta*) of either sex weighing 4 to 5 kg were used in these experiments. The monkeys were maintained, quarantined for 3 weeks, and tuberculin tested at the primate center of the Central Drug Research Institute, Lucknow, India. Monkeys which did not show any hemoprotozoan infection when tested by examination of blood smears were used in the present study.

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Process of infection. *P. cynomolgi* B was maintained at the Central Drug Research Institute by a passage through the *Anopheles stephensi* mosquito-monkey cycle (16, 17). The sporozoites harvested from *P. cynomolgi* B-infected mosquitos were maintained under standard laboratory conditions and were used for intravenous inoculations of monkeys. Giemsa-stained blood smears were examined from day 7 of infection for the appearance of patent infection in monkeys from treated and control groups. Monkeys which had been inoculated with sporozoites and in which infection did not become patent during a 60-day observation period were considered cured.

For the study of blood-induced malaria, the parasites from sporozoite-induced infection which had become patent were diluted with citrated blood and injected intravenously to give an inoculum of 10^5 trophozoites. Blood smears of the infected monkeys were examined daily for periods up to 2 to 3 weeks, and the parasitemia was monitored in both the control and treated groups.

Interferon. The production, purification, and characterization of rHuIFN-γ used in these studies was carried out at Genentech Inc. The interferon was produced in *Escherichia coli* and was greater than 98% pure as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (4). The amino acid sequence of the rHuIFN-γ employed was identical to that of natural HuIFN-γ and thus lacked Cys-Tyr-Cys amino acid residues at the NH_2 terminus (1). Titers of rHuIFN-γ were determined in virus-induced cytopathologic effect inhibition assays with human (A549) cells challenged with encephalomyocarditis virus. The assays were standardized against the International Reference preparation of rHuIFN-γ (Gg 23-901-530). The specific activity of the preparation used was approximately 1.2×10^7 IU/mg of protein. The freeze-dried rHuIFN-γ was reconstituted in sterile distilled water at the time of administration. The intramuscular route of administration was chosen because the half-life of rHuIFN-γ given by this route was longer than that given by the intravenous route in clinical trials in humans (11); intramuscular administration of rHuIFN-γ effectively protected rhesus monkeys against virus infections (J. Morrill and C. Czarniecki, unpublished results). The doses of interferon used in the present work were equivalent to the doses that proved effective in the latter study.

TABLE 1. Inhibitory effect of HuIFN-γ on sporozoite-induced *P. cynomolgi* malaria[a]

rHuIFNγ dose[b] (mg/kg per day)	No. of animals	Pre-patent period (days)	Trophozoites on day 16 after infection
0.1	4	60[c]	0
0.01	1	60[c]	0
	1	27[d]	0
	2	13, 13	600, 1,300
0.005	4	13, 14, 14, 15	1,040, 540, 250, 180
0	4	10, 10, 10, 11	10,000, 10,100, 10,100, 6,000

[a] Sporozoites (1.8×10^5) were inoculated intravenously on day 0.
[b] rHuIFNγ was injected intramuscularly on days −1 to +13.
[c] No parasitemia seen up to 60 days after infection, when the experiment was terminated.
[d] Animal died on day 27 with no sign of malaria.

RESULTS

Three interferon doses (0.005, 0.01, and 0.1 mg/kg [body weight] per day) were injected by the intramuscular route for 15 consecutive days, the first dose at 24 h before infection, the second dose at the time of infection, and the next 13 doses every 24 h. The treatment with 0.1 mg/kg per day completely suppressed infection with sporozoites in animals monitored for 60 days (Table 1). In animals receiving 0.01 mg/kg per day, parasitemia appeared in erythrocytes in two of four animals on day 13. One animal remained free of infection for the full 60-day course of the study, whereas another died on day 27 with no sign of malaria. The two animals developing parasitemia had lower levels of infection than controls on day 16 after inoculation.

In another group of animals receiving 0.005 mg/kg per day, infection in the blood was first found 13 to 15 days after inoculation of sporozoites, whereas in the controls not treated with rHuIFN-γ erythrocytes were found to contain malaria parasites 10 to 11 days after their inoculation, a regular finding in this system (Table 1). Levels of the parasite in the blood were lower at 16 days after infection in the animals treated with 0.005 mg of rHuIFN-γ per kg per day than in controls.

In another study animals were treated with 0.15 mg of rHuIFN-γ per kg per day from days −5 to +7 with respect to intravenous inoculation of *P. cynomolgi* sporozoites. In the animals receiving this schedule of treatment parasites were not seen in the blood for the 60-day course of the study, whereas in control animals not receiving rHuIFN-γ *P. cynomolgi* was found as usual on days 10 and 11 (data not presented).

A dose of 0.1 mg/kg per day given from days −4 to +6 in animals inoculated with *P. cynomolgi* trophozoites from parasitized rhesus monkey erythrocytes had no effect on the course of the infection. Four rHuIFN-γ-treated and two untreated animals developed parasitemia 6 or 7 days after intravenous inoculation of parasites.

DISCUSSION

The present studies are the first report that rHuIFN-γ can effectively protect rhesus monkeys from infection with *P. cynomolgi*. The response was dose dependent. Protection was observed for sporozoite-induced infection, but not for trophozoite-induced infection, suggesting that the inhibition occurred at the pre- or exoerythrocytic phase of the parasitic cycle in hepatocytes. Previous studies with crude interferon in mice (5–8) suggested similar conclusions. Since interferons appear to be active at this point, they may have a place in the treatment of forms of malaria where relapse due to exoerythrocytic growth is common. Of the commonly used antimalarial drugs, only primaquine is effective on this stage of parasite development; however, the toxicity of this drug especially in pregnancy, infants, and glucose-6-phosphate dehydrogenase-deficient individuals limits its general usage (20). Although maximal tolerated doses of rHuIFN-γ have been determined for cancer patients (11, 12), it is not known yet whether doses that may be effective in treating malaria in humans have acceptable levels of toxicity.

Interferons may prove to be effective in the treatment of infectious diseases by a wide range of pathogenic organisms. The antiviral effects of the alpha, beta and gamma interferons have been widely studied. Early reports utilizing crude and semipurified natural interferon preparations indicated inhibitory activity against other nonviral organisms such as rickettsiae (9, 21, 22), chlamydiae (2, 19), bacteria (3), and protozoa such as *Toxoplasma gondii* (14, 15). More recent studies with purified *E. coli*-derived rHuIFN-γ preparations have extended the range of inhibitory activities to other pathogens such as *Listeria monocytogenes* (10), *Leishmania donovani* (13), and in this study to *Plasmodium cynomolgi*.

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