

## Inhibition by Cyclosporin A of Rodent Malaria In Vivo and Human Malaria In Vitro

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The development and course of normally lethal parasitemias in mice inoculated intraperitoneally with erythrocytic stages of *Plasmodium yoelii* or *Plasmodium berghei* were markedly affected by treatment with the antilymphoid drug cyclosporin A (CS-A). When the first of four daily subcutaneous 25-mg/kg doses of CS-A was given at the time of parasite inoculation, patent infections failed to develop. If begun up to 5 days earlier, this same treatment regimen prolonged the prepatent period, attenuated parasitemia, and reduced mortality. In mice with patent infections, two consecutive daily 25-mg/kg doses of CS-A were sufficient to terminate parasitemias which, after several days, reappeared but were self-limiting. This pattern of apparent cure followed by transient recrudescence remained unaltered even when daily treatment with the same drug dose was continued for 3 weeks. Recrudescence was associated with the emergence of parasite populations that were relatively resistant to CS-A and, in the case of *P. yoelii*, of reduced virulence. In more limited experiments, CS-A was found to be active in vitro against erythrocytic stages of the human malarial parasite *Plasmodium falciparum*. Depending on the concentration of drug in the culture medium, parasite growth was either prevented or inhibited.

In 1972, Borel et al. reported that a metabolite of the fungus *Tolypocladium inflatum* inhibits antibody production in mice (5). The active component, cyclosporin A (CS-A), is a hydrophobic cyclic undecapeptide (22) capable of suppressing the development of humoral and cell-mediated immune responses in a number of mammalian species, including humans (6, 9, 10, 13, 23, 31). In contrast to other immunosuppressive drugs which destroy many types of cells within the lymphoid compartment, the primary action of CS-A is against thymus-derived (T) lymphocytes (12, 20, 34) and is reversible (3, 4, 35). Since it is well established that, in the rodent model of malaria, T lymphocytes play a central role in the development of protective immunity (7, 11, 16, 24, 33), we treated mice with CS-A initially to determine whether their susceptibility to infection with erythrocytic stages of *Plasmodium yoelii* or *Plasmodium berghei* would be enhanced. Surprisingly, the opposite effect was observed. CS-A delayed or prevented the development of parasitemia. This observation provided the basis for the present study, in which the antimalarial potential of the drug was explored in more detail both in vivo and in vitro. As reported here, CS-A exhibited a potent activity against *P. yoelii* and *P. berghei*

when administered to mice either prophylactically or therapeutically. Furthermore, CS-A prevented or markedly inhibited the growth of *Plasmodium falciparum* in cultured human erythrocytes.

### MATERIALS AND METHODS

**Mice.** Outbred ICR female mice, 6 to 8 weeks of age, were purchased from Blue Spruce Farms, Altamont, N.Y., and used in all experiments about 1 to 2 weeks after their receipt.

**Irradiation.** For some experiments, mice received lethal whole-body irradiation (850 R) at a rate of 120 R/min in a Gammacell 40 <sup>137</sup>Ce irradiation unit (Atomic Energy of Canada, Ltd., Ottawa, Ontario, Canada).

**Experimental infections.** The lethal (L) and nonlethal (NL) biotypes of the 17X strain of *P. yoelii* were obtained originally from Charles Evans, Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Based on cloning procedures previously described (32), *P. yoelii* (NL) was passaged twice in mice at limiting dilutions to maintain its NL characteristic. The resulting parasite population was assumed, but not proven, to be cloned. The chloroquine-sensitive NK65 strain of *P. berghei* was provided by James Murphy, University of Maryland School of Medicine, Baltimore. Stocks of each of these organisms were stored in liquid nitrogen as a 30% suspension of parasitized blood in Hanks balanced salt solution containing 10% (vol/vol) glycerin.

TABLE 1. Modification or prevention of lethal *P. yoelii* and *P. berghei* infections with CS-A

Infecting parasite	CS-A doses, days 0-3 (mg/kg) <sup>a</sup>	Mean interval (days) to parasitemia of:		P/T <sup>b</sup>	D/T <sup>b</sup>	Mean survival time $\pm$ SEM (days)
		0.5-1.0%	$\geq$ 25.0%			
<i>P. yoelii</i>	None	3	7	5/5	5/5	13.0 $\pm$ 1.6
	1	4	9	5/5	4/5	13.3 $\pm$ 2.3
	5	10	16	5/5	4/5	20.5 $\pm$ 1.9
	25			0/15	0/15	
<i>P. berghei</i>	None	3	16	10/10	10/10	20.2 $\pm$ 1.4
	25	15	26	1/10	1/10	36

<sup>a</sup> Four daily doses of CS-A were given s.c. beginning at the time of i.p. infection (day 0) of groups of mice with 10<sup>6</sup> *P. yoelii* (L)- or *P. berghei*-parasitized erythrocytes.

<sup>b</sup> Portion of total mice per group (T) that developed parasitemia (P) and died (D).

Before use, they were passaged once in mice from which blood was obtained during rising parasitemias and used to produce experimental infections. Except as noted, all infections were initiated by inoculation intraperitoneally (i.p.) of 10<sup>6</sup> parasitized erythrocytes. Levels of parasitemia were determined at appropriate intervals by microscopic examination of Giemsa-stained films of tail blood. Usually, the number of infected erythrocytes in a total of approximately 500 was counted and expressed as a percentage. When parasitemias were less than 3%, approximately 1,500 cells were examined. The FCR-3/Gambia strain, subline D, of *P. falciparum* (18) was grown in vitro (30) in 6% human erythrocyte suspensions (vol/vol) as described previously (26, 27).

**Preparation and use of CS-A.** CS-A was provided by Jean Borel, Sandoz Ltd., Basel, Switzerland. For use in treating mice, 100 mg of drug was dissolved in 2 ml of Emulphor EL620 (kindly supplied by the GAF Corp., New York, N.Y.), and distilled water was added to give the desired final concentration. Either CS-A or diluent alone (as a control) was administered subcutaneously (s.c.) to mice in volumes ranging from 0.1 to 0.15 ml. For use in vitro, CS-A was dissolved in dimethyl sulfoxide, certified spectroanalyzed (Fisher Scientific Co., Pittsburgh, Pa.), to give a concentration of 80 mg/ml, and 50  $\mu$ l of this stock solution was added to 40 ml of culture medium. Control cultures contained equal quantities of dimethyl sulfoxide only, and all experiments included a normal medium control without dimethyl sulfoxide. Stock solutions were sterilized by filtration and diluted to the desired concentrations with sterile medium.

## RESULTS

**Prevention or attenuation of murine malaria with CS-A.** After relatively constant prepatent periods, control mice inoculated with *P. yoelii* (L) or with *P. berghei* developed parasitemias which rose progressively and were uniformly fatal. The mean survival times post-inoculation were 13 and 21 days, respectively. In contrast, detectable parasitemias consistently failed to develop in mice given four consecutive daily doses (25 mg/kg) of CS-A beginning at the time of parasite inoculation (day 0) (Table 1). It should be noted that in these experiments, larger

doses of CS-A were not used in order to minimize the immunosuppressive effect of the drug. Smaller doses (1 or 5 mg/kg) prolonged the prepatent period and occasionally attenuated parasitemia and prevented death. Drug-treated mice in which patency failed to occur remained fully susceptible to challenge with the same organism several weeks later, whereas recovered mice were completely resistant to reinfection (data not shown).

Groups of mice were given four daily 25-mg/kg doses of CS-A at different times before inoculation with *P. yoelii* (L) to determine the duration of the prophylactic effect of the drug. This effect was short-lived (Table 2). Mice treated with the fourth dose 2 days (day -2) or more before infection were as susceptible as untreated mice. After a prolonged prepatent period, both fatal and nonfatal malaria occurred in groups of mice given the fourth dose of CS-A either 1 day before or at the time of infection, or 2 days after infection (day 2). Only when treatment was initiated concurrently with parasite inoculation

TABLE 2. Prophylactic efficacy of CS-A treatment begun at different times before infection with *P. yoelii* (L)

Day of last CS-A dose (25 mg/kg) <sup>a</sup>	Mean prepatent period (days)	Mean interval to 25% parasitemia (days)	P/T <sup>b</sup>	D/T <sup>b</sup>
-4	1	4	4/4	4/4
-2	1	4	4/4	4/4
-1	2	9	4/4	2/4
0	6	14	4/4	2/4
2	9	13	2/4	2/4
3			0/4	0/4

<sup>a</sup> Four daily doses of CS-A were given s.c. to groups of mice at various times before i.p. infection with 10<sup>6</sup> *P. yoelii* (L)-parasitized erythrocytes on day 0.

<sup>b</sup> Portion of total mice per group (T) that developed parasitemia (P) and died (D).

TABLE 3. Failure of lethal irradiation to prevent inhibition of *P. yoelii* infection by CS-A

CS-A dose, days 0-2 (25 mg/kg) <sup>a</sup>	Irradiation (850 R)	Mean interval (days) to parasitemia of:		P/T <sup>b</sup>	D/T <sup>b</sup>	Mean survival time ± SEM (days)
		0.5-1.0%	≥25.0%			
+	+			0/5	5/5	12.5 ± 0.5
-	+	3	7	5/5	5/5	9.4 ± 0.4
+	-	10	15	3/5	2/5	20.5 ± 1.5
-	-	1	4	5/5	5/5	6.8 ± 0.9

<sup>a</sup> Three daily doses of CS-A were given s.c. to groups of mice beginning at the time of infection (day 0) with  $10^6$  *P. yoelii* (L)-parasitized erythrocytes.

<sup>b</sup> Portions of total mice per group (T) that developed parasitemia (P) and died (D).

(last dose on day 3) were all mice permanently prevented from developing parasitemia.

To insure that the antimalarial activity of CS-A was not an indirect result of drug-induced immunostimulation (19), CS-A was tested for its ability to prevent or modify *P. yoelii* (L) parasitemia in lethally irradiated (850 R) mice. It had been determined in previous experiments that although ablation of hemopoiesis by lethal irradiation lengthened the prepatent period, this effect could be overcome in part by increasing the infecting dose of parasites. Thus, 3 h after being irradiated, 10 mice were inoculated i.p. with  $5 \times 10^6$  erythrocytes parasitized with *P. yoelii* (L), and 1 h later, half were given the first of three daily 25-mg/kg doses of either CS-A or diluent. For comparison, 10 normal mice were infected and treated in the identical fashion. Irradiated mice were prevented from developing detectable parasitemia by CS-A but eventually died from bone marrow aplasia (Table 3); those untreated all developed fatal malaria. Three of five normal mice treated with three doses of CS-A (instead of four as previously) became parasitemic after a considerably longer prepatent period than occurred in those untreated. Of the former, parasitemia was self-limiting in one mouse that survived but unusually protracted in the two that died about 3 weeks postinfection.

**Effect of CS-A on patent infections.** Having found that murine malaria could be prevented with CS-A, we next explored the potential of the drug to cure existing parasitemia produced by *P. yoelii* (L), *P. yoelii* (NL), and *P. berghei*. Initially, 15 mice were inoculated with *P. yoelii* (L), and 6 days later (day 6), when the average level of parasitemia was about 10%, each of 5 mice was given CS-A (25 mg/kg) followed by a second identical dose 24 h later. Five more mice were subjected to the same treatment schedule beginning on day 8, when their parasitemias averaged about 30%. All mice continued to be monitored individually for changes in parasitemia which, in those untreated, rose progressively until death occurred between days 9 and 17 (Fig. 1). In

contrast, parasitemias fell sharply in all treated mice, becoming undetectable within a 2-day period after the first dose of CS-A. It should be noted that during the first 24 h of this period, the most prominent change in the blood of these mice was a selective reduction in ring forms. However, this apparent "cure" was short-lived, lasting up to 5 days. Parasitemia then reappeared and persisted at relatively low levels for about another 5 days before becoming undetectable (Fig. 1). In three similar experiments, this sequence of temporary cure, recrudescence, and permanent cure was seen in virtually all *P. yoelii* (L)-infected mice that were first treated with CS-A up to 6 days after patency became detectable, even when the treatment period was extended from 2 days to several weeks. Interestingly, patent *P. yoelii* (NL) infections were also

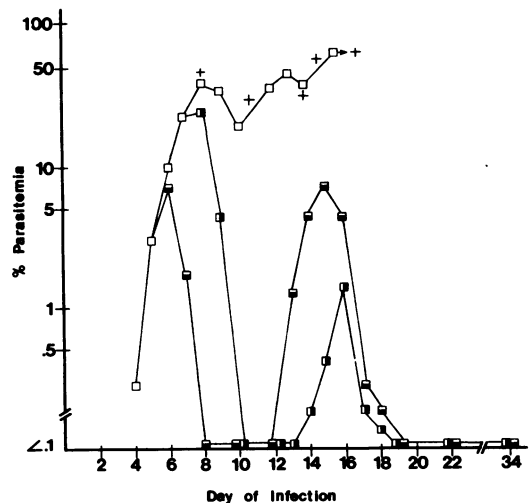


FIG. 1. Course of parasitemia in mice treated with two consecutive doses of CS-A (25 mg/kg) during patent *P. yoelii* (L) infections. Mice (in groups of five) were given CS-A on either days 6 and 7 (■) or days 8 and 9 (□) postinfection. Deaths (+) occurred only in infected control mice given drug diluent on days 6 through 9 (□). Each point represents the geometric mean parasitemia per group.

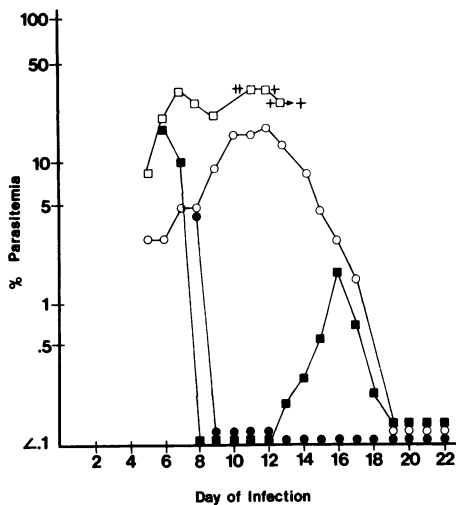


FIG. 2. Course of parasitemia in mice treated chronically with CS-A (25 mg/kg per day) during patent *P. yoelii* (L) and *P. yoelii* (NL) infections. Five *P. yoelii* (L)-infected mice were given either CS-A (■) or drug diluent (□) on days 6 through 21 postinfection. Five *P. yoelii* (NL)-infected mice were given either CS-A (●) or drug diluent (○) on days 8 through 21 postinfection. +, Deaths. Each point represents the geometric mean parasitemia per group.

abruptly terminated by CS-A treatment but failed to recrudescence. These points are illustrated by the results of the experiment depicted in Fig. 2. Beginning on days 6 and 8 post-inoculation with, respectively, *P. yoelii* (L) and *P. yoelii* (NL), mice were treated daily with CS-A (25 mg/kg) through day 21; recrudescence occurred only in *P. yoelii* (L)-infected mice, all of which survived and were immune (data not shown) to subsequent reinfection. In untreated control mice, *P. yoelii* (L) was uniformly lethal whereas self-limiting infections by *P. yoelii* (NL) remained patent about 10 days longer than in CS-A treated mice.

Recrudescence parasitemia was also consistently found when patent *P. berghei* infections were aborted by CS-A treatment (25 mg/kg per day) lasting 2 days or more. Treatment of five mice for 2 days beginning on day 14 postinfection caused their parasitemias to fall rapidly to undetectable levels (Fig. 3). Parasitemias reappeared in all mice 2 to 3 days later, and only two survived. Of five mice continuously treated from days 14 through 40, two recrudesced after remaining subpatent for nearly 2 weeks; parasitemia persisted at relatively low levels for about another 2 weeks, but these animals survived.

**Emergence of parasites resistant to CS-A.** The transient reappearance of parasitemia consistently seen in mice undergoing either short-term or continuous treatment with CS-A (Fig. 2 and 3)

suggested that the drug was selecting for the growth of resistant parasites whose subsequent clearance was immune mediated. The results of several experiments indicated that this was the case since CS-A could not prevent infections in recipients of blood obtained during recrudescing *P. yoelii* (L) *P. berghei* parasitemias. Representative data from one such experiment are shown in Table 4. Four groups of mice were infected with  $10^6$  parasitized erythrocytes from the blood of a single mouse in which *P. yoelii* (L) parasitemia recrudesced during continuous drug treatment. Beginning immediately after infection, one group (controls) received drug diluent, and the others were given CS-A in doses of 25, 50, or 100 mg/kg at daily intervals. In the control group, CS-A-resistant *P. yoelii* (L)—hereafter designated *P. yoelii* (R)—produced parasitemias that were lethal for eight mice and self-limiting in the remaining two. By comparison, all 10 mice treated with 25 mg of CS-A per kg exhibited parasitemias that rose more slowly, persisted longer, and (except in one animal) were self-limiting. The development and duration of parasitemia were even more prolonged in the two groups of mice treated with the two larger CS-A doses. Except for the self-limiting infection that occurred in two mice from the 50-mg/kg group, parasitemia was eventually lethal for the remaining animals in these groups.

These results (Table 4) suggested that *P. yoelii* (R) was less virulent than *P. yoelii* (L), since

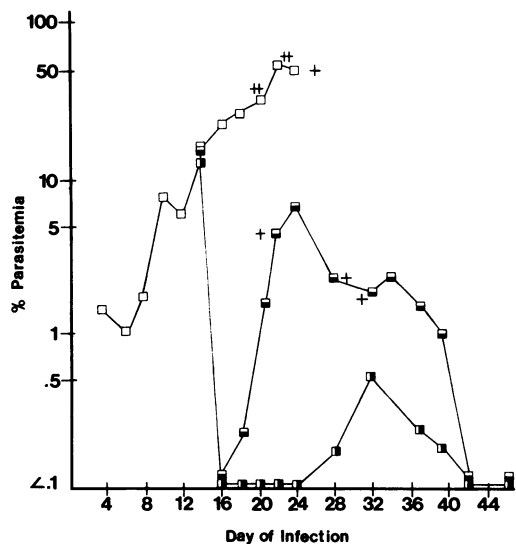


FIG. 3. Course of *P. berghei* parasitemia in five mice given CS-A (25 mg/kg per day) on either days 14 and 15 (■) or days 14 through 40 (□) postinfection and in five untreated control mice (□). +, Deaths. Each point represents the geometric mean parasitemia per group.

TABLE 4. Effect of continuous treatment with different doses of CS-A on *P. yoelii* (R) infections

Daily CS-A dose (mg/kg)	Treatment period <sup>a</sup> (days)	Mean prepatent period (days)	Mean interval to 10% parasitemia (days)	Self-cures <sup>b</sup>	Mean survival time $\pm$ SEM (days)
None		2	5	2/10	14.1 $\pm$ 1.1
25	0-26	2.5	11	9/10	19.0 $\pm$ 0.7
50	0-32	3	18	2/6	27.8 $\pm$ 1.5
100	0-32	4	20	0/5	28.8 $\pm$ 2.7

<sup>a</sup> Mice were given CS-A s.c. daily beginning at the time of i.p. infection with  $10^6$  *P. yoelii* (R)-infected erythrocytes.

<sup>b</sup> Mice in which parasitemia eventually became undetectable/total mice in group.

without exception the latter had always produced lethal infections (>300 mice tested), and also that the resistance of *P. yoelii* (R) to CS-A was not absolute, since daily treatment with all three doses of the drug inhibited its rate of growth. At the lowest dose (25 mg/kg), this inhibition was sufficient to permit the majority of mice to clear their infection, presumably through the development of specific immunity. On the other hand, the greater decrease in parasite growth rates in mice treated with 50 and 100 mg of CS-A per kg was probably outweighed by the immunosuppressive and toxic effects of the drug, thereby resulting in a net prolongation of infection and ultimately in death.

The reduced lethality of *P. yoelii* (R) and its resistance to CS-A remained unchanged after two additional blood passages in mice. Furthermore, *P. yoelii* (R) showed no detectable change in its host cell preference, and like *P. yoelii* (L), it grew in both mature and immature erythrocytes.

As mentioned above, essentially similar findings were obtained with *P. berghei*, except that infections with drug-resistant parasites continued to be uniformly lethal (data not shown).

**Inhibition by CS-A of *P. falciparum* growth in vitro.** The results of the previous experiments provided the rationale for determining whether

CS-A could prevent or suppress in vitro the growth of the human malarial parasite *P. falciparum*. As in previous studies of other potential antiparasitodal compounds (26, 27), the conventional candle jar technique (17) was employed to propagate *P. falciparum* in cultured human erythrocytes. In two separate experiments, cultures of erythrocytes, of which 0.4% were infected with *P. falciparum*, were established initially in the absence of CS-A and maintained for 24 h. At the end of this period and thereafter at 24-h intervals, the culture medium was replaced with that containing either CS-A or (in control cultures) a drug diluent. The final concentrations per ml of CS-A ranged from 100  $\mu$ g ( $8.32 \times 10^{-5}$  M) to 0.01  $\mu$ g in 10-fold decrements. After the first 24 h of exposure to drug, the levels of parasitemia in all cultures were determined each time the medium was replaced. These experiments yielded very similar results and showed that CS-A inhibited *P. falciparum* growth in a dose-dependent fashion. The data from one experiment are presented in Table 5. No parasites were detectable in erythrocytes from cultures containing 100  $\mu$ g of CS-A per ml; at 10  $\mu$ g/ml, parasitemia was markedly depressed after 48 h of drug exposure and then disappeared. Lower drug concentrations, although failing to eliminate parasitized cells, caused significant reductions in their numbers during the remainder of the 5-day culture period.

In another experiment, medium containing 1  $\mu$ g of CS-A per ml ( $8.32 \times 10^{-7}$  M) was added to 24-h-old cultures of *P. falciparum*-infected erythrocytes as described above. However, CS-A was omitted from all subsequent changes of medium, so that the cells were exposed to the drug only during day 2 of the total culture period. Under these conditions, parasite growth was inhibited during days 3, 4, and 5 (as compared with controls) by, respectively, 34.6, 48.8, and 29.0%. The decreased inhibition seen on the last day provided some indication that the effect of drug might be reversible. This would be compatible with the very small number of cells

TABLE 5. Inhibition by CS-A of *P. falciparum* growth in vitro

CS-A concn ( $\mu$ g/ml)	% Reduction in parasitemia at <sup>a</sup> :			
	48 h	72 h	96 h	120 h
100	100	100	100	100
10	0	69	100	100
1	0	46	60	66
0.1	0	43	22	22
0.01	0	0	0	22

<sup>a</sup> Each value (average of three slides) is based on parasitemias existing in corresponding control cultures containing drug diluent. Mean parasitemias in control cultures were: 0.8% (48 h), 1.3% (72 h), 3.2% (96 h), and 4.6% (120 h).

containing abnormal-appearing parasite stages. The extent of *P. falciparum* inhibition in companion cultures maintained continuously in the presence of the same CS-A concentration (data not shown) was comparable to that observed previously (Table 5).

## DISCUSSION

This study was initiated as a result of the seemingly paradoxical observation that the immunosuppressive drug CS-A inhibited rather than potentiated plasmodial infections of mice. The results presented above have clarified this observation by demonstrating that the antimalarial activity of CS-A is separable from its well-documented effects on the immune system, although the mechanism by which this activity is expressed is obscure. Previous investigations employing mice and other species of laboratory animals provide no indication that the function(s) of cells other than mature T lymphocytes is affected by regimens of CS-A similar to those used in the present study. Larger doses of drug or more prolonged treatment periods are necessary to induce toxic side effects. For example, in rats, daily administration of 45 mg of CS-A per kg for a period of 13 weeks was required to cause reproducible histological alterations in lymphoid, hepatic, and renal tissues (4).

One possible clue to the manner in which CS-A may prevent or terminate patent infection is the strikingly selective reduction in ring stages of *P. yoelii* (L) that was seen 24 h after CS-A treatment of parasitemic mice—an observation that also applied to *P. berghei* (J. Murphy, unpublished data). In the apparent absence of any concomitant morphological abnormalities of trophozoite or schizont stages, this observation may reflect an ability of CS-A to interrupt the growth cycle of the parasites by preventing merozoites from invading erythrocytes. Theoretically, this could result either from a direct effect of the drug on merozoites or from some alteration of erythrocytes in general which prevents their invasion. The latter situation seems unlikely, however, since any significant metabolic or structural alteration of erythrocytes induced by CS-A in vivo would be expected to induce a detectable compensatory change in the existing hematological picture. We, as well as others (29), have not observed such a change in drug-treated normal animals.

There is relatively little published information as to the distribution of cell types which preferentially bind CS-A. Of those in the blood, lymphocytes, monocytes, and neutrophils, but not erythrocytes, appear to possess distinct CS-A-binding sites of high affinity (25). On the other hand, the outer membranes of *Plasmodium*-

infected erythrocytes are known to exhibit an increased lipid fluidity (15), which might possibly favor the selective binding of CS-A because of its strong lipophilic property.

It appears that the susceptibility to CS-A of *P. yoelii* or *P. berghei* in vivo and that of *P. falciparum* in vitro are comparable. The average weight of mice used in this study was 20 g; therefore, at a dose of 25 mg/kg, each received approximately 500  $\mu$ g of CS-A. Since probably less than 50% of the administered drug enters the intravascular compartment and most of it is metabolized in the liver within 24 h (2), its concentration in the blood almost certainly did not exceed 50  $\mu$ g/ml (assuming a blood volume of 5 ml) during preventive or curative treatment—periods of 4 and 2 days, respectively (Fig. 1 and 3). In a group of mice treated with four daily 5-mg/kg doses of CS-A beginning on the day of *P. yoelii* (L) infection, blood levels of the drug probably averaged no more than 10  $\mu$ g/ml, yet they were sufficient to cause a marked delay in the appearance of parasitemia and to allow one animal to survive (Table 1). Similarly, CS-A concentrations of 100 and 10  $\mu$ g/ml were capable of, respectively, preventing and terminating *P. falciparum* infection in vitro (Table 5).

There is no clear-cut explanation for the regularity with which CS-A treatment of patent *P. yoelii* (L) and *P. falciparum* infections led to the emergence of drug-resistant parasites. Two major possibilities exist. CS-A selected for their growth from either (i) a mixture of sensitive and resistant populations or (ii) spontaneously arising mutant populations, one or more of which were resistant (1). At the present time, we favor the former possibility for the following reasons. First, preliminary findings suggest that when the inoculum size of *P. yoelii* (L) is increased from  $10^6$  to  $10^8$  parasitized erythrocytes, the prevention of patent infection with CS-A becomes irregular, and initial breakthrough parasitemias appear to be drug resistant. Second, it seems more than coincidental that parasitemias produced by *P. yoelii* (NL), which underwent a standard cloning procedure, did not recrudescence after being cured with CS-A (Fig. 2) as did uncloned *P. yoelii* (L) and *P. berghei* (Fig. 1 and 3). Third, assuming that most, if not all, recrudescence infections were due to drug-resistant parasites, it is difficult to envisage how they could have appeared so rapidly in the more than 60 mice thus far examined without having been present in the original inocula.

The resistance of *P. yoelii* (R) to CS-A as well as its reduced virulence made it possible to observe more readily the effect of drug treatments of different immunosuppressive potentials. In a previous study of the ability of CS-A to suppress the cellular immune responses of

mice to vaccinia and lymphocytic choriomeningitis viruses (G. A. Cole and S. P. Nickell, unpublished data), we found that if begun 1 day before virus priming, daily treatment with s.c. doses of 100 or 50 mg/kg, but not 25 mg/kg, completely prevented the induction of specific cytotoxic T lymphocytes by either virus. Thus, both the course and the outcome of *P. yoelii* (R) infections initiated concurrently with daily CS-A treatment were markedly dependent upon drug dose (Table 4). Mice that received the lowest T lymphocyte-suppressive dose (25 mg/kg) fared better than those untreated; although the development of parasitemia was prolonged, there was a significant increase in the number of self-cures. It seems, therefore, that *P. yoelii* (R) retained some sensitivity to CS-A which favored, presumably, its eventual immune-mediated elimination. Repeated doses of 50 or 100 mg/kg prolonged the development of parasitemia even more but, at the same time, probably impaired or prevented the induction of antimalarial immunity.

In addition to this study, we are aware of two others in which CS-A has been reported to have an antiparasitic effect. Bueding et al. (8), while investigating the potential of CS-A to depress the immune-mediated development of liver granulomata in *Schistosoma mansoni*-infected mice, found unexpectedly that five daily 25-mg/kg doses strikingly inhibited all stages of worm development. Furthermore, it appeared that a synergistic effect was obtained when CS-A was used in combination with the new antischistosomal drug amoscanate. Three daily 25-mg/kg doses of CS-A produced marked reductions in both the hemoglobinase activity and the protein content of adult schistosomes, particularly females, which persisted for more than 2 weeks after the cessation of treatment. Although these findings suggested the possibility that the antimalarial activity of CS-A might involve a similar mechanism, attempts to demonstrate a decreased protease (or hemoglobinase) activity of *P. falciparum* after treatment in vitro with CS-A have been unsuccessful (F. N. Gyang and L. W. Scheibel, unpublished data).

More recently, Thommen-Scott has described an inhibitory effect of orally administered CS-A on *P. berghei* and *Plasmodium chabaudi* infections of mice (28). Although basically in agreement with our findings, the results of this study were more limited and obtained under somewhat different experimental conditions. Interestingly, however, they indicated that CS-A may synergize with pyrimethamine, a drug which seems to affect mainly schizont stages (14, 21).

From the available evidence, including that presented in this paper, it appears that CS-A may represent a new class of compounds having

potential application to the treatment of malaria and possibly of other parasitic infections. Although it shares with other antiplasmodial compounds the ability to induce the emergence of resistant parasites, CS-A or one of its congeners might be used most successfully in combination with other therapeutic drug regimens and in quantities insufficient to depress the immune system. In this context, we have been encouraged by the preliminary finding (J. Murphy, unpublished data) that resistant populations of *P. berghei* which arose during CS-A treatment of patent infections retain their susceptibility to chloroquine.

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