Antischistosomal Effect of Cyclosporin A: Cure and Prevention of Mouse and Rat Schistosomiasis Mansoni

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C57BL/6 mice infected with *Schistosoma mansoni* at day 0 and injected with cyclosporin A (CyA) either daily or from day −1 to day 3 were protected against schistosomiasis mansoni as indicated by a decrease in the number of worms recovered from the liver 45 days after infection. CyA treatment also protected rats and strains of mice with known immunity defects (*nu/nu*, P/N, CBA/N). Protection was evident against both primary and secondary infection in mice infected at day 0, reinfected at day 42, and treated daily with CyA either during the course of the experiment or only from day −1 to day 3, as indicated by the worm burden at day 67. In such an experiment of infection and reinfection, the immature worms were shown to be the target of CyA. Administration of the drug 27, 45, 62, or 100 days before infection confirmed the long-term protective effect of CyA. This drug did not evoke the killing of adult worms in vivo. These data confirm and define the curative and preventive effect of CyA against schistosomiasis mansoni.

Cyclosporin A (CyA) has recently been shown by us (5) and others (6, 13) to protect mice against schistosomiasis. CyA is a valuable drug in human organ transplantation, mainly because of its ability to prevent the rejection of HLA-mismatched allografts (7). In animal models, CyA has been found to preferentially inhibit T-lymphocyte-mediated immune responses while not affecting B lymphocyte or macrophage effector functions (3, 18).

In murine schistosomiasis, adult worms resulting from a primary infection are unaffected by the host response, even though they induce resistance to reinfection, or concomitant immunity (16). We first used CyA to test the involvement of specific immune mechanisms in the resistance to reinfection by schistosomes. Surprisingly, it was observed that, after treatment with CyA, mice are protected against both primary and secondary infection with *Schistosoma mansoni* (5). To further define the effect of CyA, the efficacy of various protocols of drug administration was evaluated. In addition, mouse strains with a variety of known immunological deficiencies were used. The rat was also examined to confirm our observations in another schistosome-infected-animal model.

MATERIALS AND METHODS

Animals. Female mice, 6 to 8 weeks of age, were used in all experiments. C57BL/6 mice were purchased from Charles River Breeding Laboratories, Inc., Paris, France; nude (*nu/nu*) and *nu/+* BALB/c mice were purchased from Centre National de la Recherche Scientifique, Orléans, France; and CBA/Ca and CBA/N mice were purchased from OLAC Ltd., Shaws Farm, Blackthorn Bicester, Oxon, England. P/N mice were bred in our facilities from breeders obtained as a gift from Monte Meltzer, National Institutes of Health, Bethesda, Md. Female Fisher rats (6 to 8 weeks old) were purchased from Charles River Breeding Laboratories.

Infection. *S. mansoni* originating from Puerto Rico was maintained in albino *Biomphalaria glabrata* snails and outbred hamsters. The cercariae were used for infection within 2 h of emergence. Animals were infected under anesthesia by the percutaneous ring method (15). Perfusion

of worms from the portal vein was performed by the method of Smithers and Terry (15). For infection-reinfection experiments, mice were infected at day 0, reinfected at day 42 or day 46, and perfused at day 67, at which time mature and immature worms, established from infection and reinfection, respectively, could easily be distinguished as described by Doenhoff et al. (8). Pulmonary perfusion was performed as described by Sher et al. (14).

Preparation and use of CyA. CyA was kindly supplied by J. F. Borel and E. Wiskott of Sandoz Ltd., Basel, Switzerland. Miglyol 812 was generously provided by Dyna France, Paris. CyA was dissolved in Miglyol 812 (6 mg/ml) and administered subcutaneously at a daily dose of 30 mg/kg of body weight, unless otherwise stated. Control animals received Miglyol 812 alone.

RESULTS

Original experiment: effect of CyA administered daily during the infection-reinfection experiment. To test the involvement of immune mechanisms with resistance to reinfection, C57BL/6 mice were infected at day 0 and reinfected at day 42 (with 60 and 100 cercariae, respectively). CyA (30 mg/kg of body weight per day) was injected from day −1 to day 67, which was the day of portal perfusion. Surprisingly, a total protection was obtained against both primary and secondary infection as compared with the respective controls (Fig. 1). Resistance to reinfection of untreated mice was 85% as compared with the challenged control mice.

Effect of CyA on infection. To assess the anti-schistosomal effect of CyA, several protocols of drug administration were tested.

(i) **Daily administration of CyA throughout the course of infection.** C57BL/6 mice (10 mice per group) were infected at day 0 with 60 cercariae, and CyA or solvent was injected from day −1 to day 45. No eggs were found upon examination of the liver by microscopy, and no worms were recovered by liver perfusion on day 45 in the CyA-treated group in comparison with the control group, from which 26 ± 4 worms (mean ± standard deviation) were recovered. Similar experiments, performed with CBA/Ca and CBA/N mice, showed similar results. No worms were recovered from treated mice, whereas the parasite burden of untreated
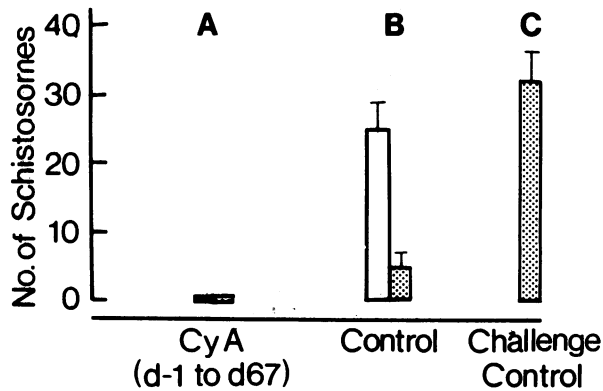
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FIG. 1. Number of worms recovered from first infection (▭) and reinfection (▨) (mean ± standard deviation) of groups of 8 to 10 C57BL/6 mice. Groups of CyA-treated (A) and control (B) mice were infected at day 0 and challenged at day 42 with 60 and 100 cercariae, respectively. Mice in group C were challenge controls. All the mice were perfused at day 67.

CBA/Ca and CBA/N mice was 24.7 ± 3.1 and 22 ± 2.1, respectively.

(ii) CyA administration from day −1 to day 3. In three separate experiments, C57BL/6 mice (8 to 10 treated or untreated mice per group) were each infected with 60 cercariae, and CyA or solvent was injected from day −1 to day 3. Portal perfusion of worms was performed on day 45. Almost complete protection was obtained in treated mice (mean worm recovery, 0.17 ± 0.4) as compared with untreated mice (mean worm recovery, 36.1 ± 0.16). In two separate experiments, nude (nu/nu) and nu/+ BALB/c mice (five treated or untreated animals per group) were treated with CyA according to the same protocol. A similar degree of protection was observed in both nu/nu (mean worm recovery, 4 ± 4.4 as compared with untreated mice, 23 ± 8.1) and nu/+ (mean worm recovery, 0.75 ± 1.2 as compared with untreated mice, 21.8 ± 7.6) mice (Fig. 2). P/N and C57BL/6 mice were also treated with CyA according to the same protocol. Protection was obtained in two separate experiments with five mice per group in both P/N (mean worm recovery, 0.14 ± 0.38 as compared with untreated mice, 36.2 ± 8.6) and C57BL/6 (mean worm recovery, 0.43 ± 1.5 as compared with untreated mice, 39.7 ± 10.6) mice.
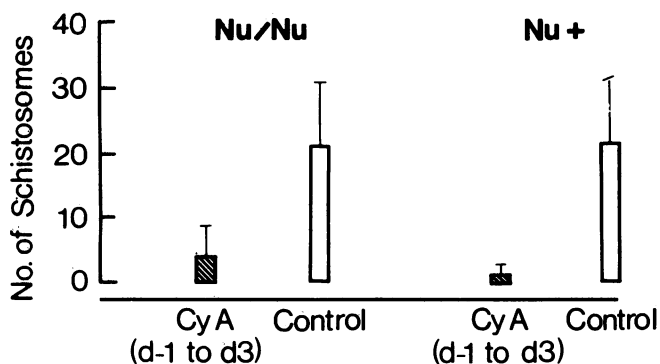
(iii) CyA administration only once. When C57BL/6 mice

FIG. 2. Number of worms (mean ± standard deviation) recovered from CyA-treated and control nude or normal mice in two different experiments with five mice per group. Mice were infected at day 0 with 60 cercariae and perfused at day 45.
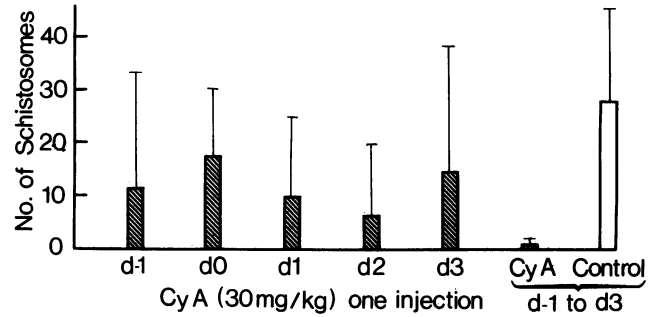

FIG. 3. Number of worms (mean ± standard deviation) recovered from CyA-treated and control C57BL/6 mice in three different experiments with a total of 8 to 10 mice per group. Mice were infected at day 0 with 60 cercariae, injected with CyA only once at day −1, day 0, day 1, day 2, or day 3 as indicated or with CyA or solvent from day −1 to day 3, and perfused at day 45.

were infected in three different experiments at day 0 with 60 cercariae and injected with CyA on day −1, day 0, day 1, day 2, or day 3, with a total of 8 to 10 mice per group, a lower but significant protection ($P < 0.01$) was obtained. The recoveries of worms on these days were 13.4 ± 18, 18.1 ± 10, 10.6 ± 12.2, 7.1 ± 10.8, and 16.4 ± 19.4, respectively (Fig. 3). As we have shown above, mice injected with CyA from day −1 to day 3, which served as control animals in this experiment, were almost totally protected (mean worm recovery, 0.23 ± 0.4) as compared with untreated mice (mean worm recovery, 31.8 ± 12; $P < 0.001$).

Effect of CyA administration from day −1 to day 3 upon infection and reinfection. Because of the lower protection obtained with only one CyA injection, as shown in the previous experiment (Fig. 3), we decided to use the protocol of five injections of CyA from day −1 to day 3 in the infection-reinfection model. In four separate experiments (10 mice per group in each experiment), C57BL/6 mice were infected at day 0 and reinfected at day 42 with 60 and 100 cercariae, respectively. Total perfusion of worms was performed at day 67. A high degree of protection was obtained not only against primary infection (96%; $P < 0.001$) but also against secondary infection (90%; $P < 0.001$), that is, 42 days
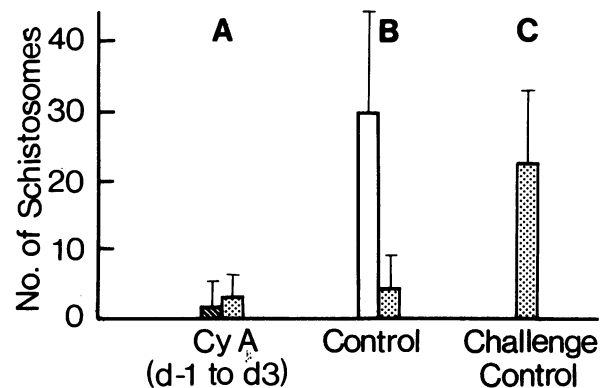

FIG. 4. C57BL/6 mice (four different experiments with 10 mice per group) in groups A and B were each infected at day 0 and challenged at day 42 with 60 and 100 cercariae, respectively. Mice in group C were challenge controls. All of the mice were perfused at day 67. The number of worms recovered from first infection (▨ and ▭) and reinfection (▨) are shown (mean ± standard deviation).
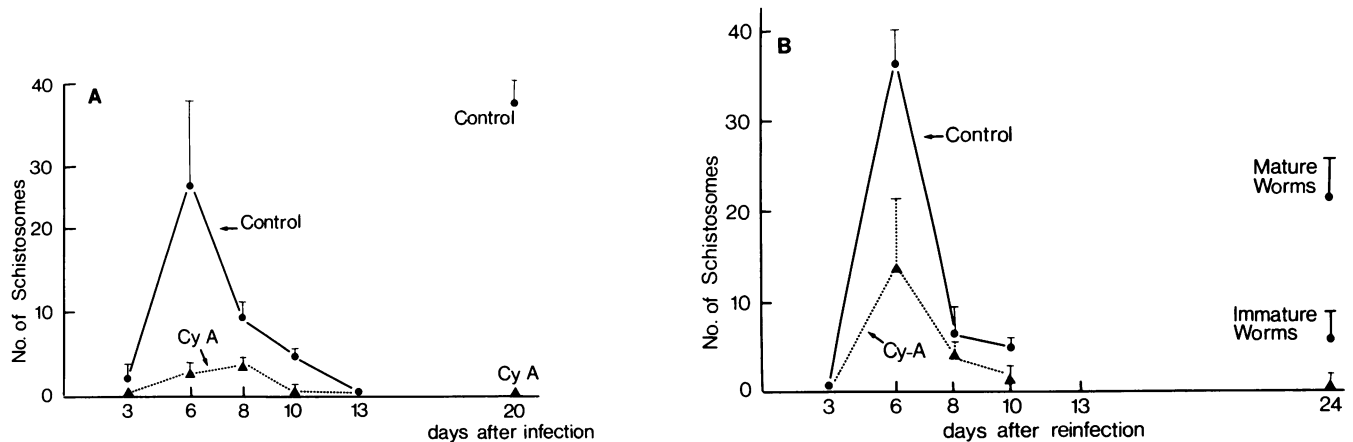
FIG. 5. Patterns of worm recovery from CyA-treated (day −1 to day 3) and control C57BL/6 mice in infection (A) or reinfection (B). Mice were infected at day 0 and reinfected at day 42 with 60 and 100 cercariae, respectively. Each point corresponds to the number of worms recovered by pulmonary or liver perfusion at day 20 or day 24 from four to seven mice.

after the last administration of CyA (Fig. 4). Resistance of untreated mice to reinfection was 79% compared with the challenged control mice.

Kinetics of elimination of worm population after CyA administration. C57BL/6 mice were infected at day 0 and reinfected at day 42 with 60 and 100 cercariae, respectively. CyA or solvent was injected from day −1 to day 3. Pulmonary perfusions of worms were performed at different times after infection and reinfection. Liver perfusions were performed at day 20 after infection and at day 24 after reinfection. For each time point, four mice were used. Elimination of the parasites by treated animals was shown to be predominantly an early event in the primary infection, as well as in the secondary infection (Fig. 5). At day 6, only 10% of the schistosomula were recovered from CyA-treated mice, compared with control mice, as determined by pulmonary perfusion. At day 20, as determined by liver perfusion, no worms were recovered from the treated mice. A similar pattern of worm elimination was shown after reinfection. At day 48, i.e., 6 days after challenge, 30% of the schistosomula were recovered from the treated mice, compared with the control animals. At day 66, i.e., 24 days after challenge, no adult

worms, and only 0.17 ± 0.4 immature worms established from reinfection, were recovered from the treated mice. In control mice, 20 ± 3.5 mature and 5.1 ± 2.1 immature worms, established from infection and reinfection, respectively, were counted.

Effect of CyA administration 27 to 100 days before infection. CyA was administered to C57BL/6 mice according to the protocol of five injections at different times before infection with 60 cercariae on day 0. In two separate experiments (10 mice per group in each experiment), CyA was injected from day −31 to day −27 before infection. In another series of experiments (10 mice per group), CyA was injected at the following times: from day −49 to day −45, from day −66 to day −62, from day −80 to day −76, or from day −104 to day −100. All of these mice were infected on day 0 and perfused at day 35 after infection. In all of these experiments, a high degree of protection was obtained (Fig. 6).

Effect of CyA administration on established adult worms. The effect of CyA on adult worms was tested by using the model of infection and reinfection. By injecting CyA (30 mg/kg of body weight per day) from day 41 to day 45 (i.e., from day −1 before challenge to day 3 after challenge) and by perfusing at day 66 (i.e., 66 days after primary infection
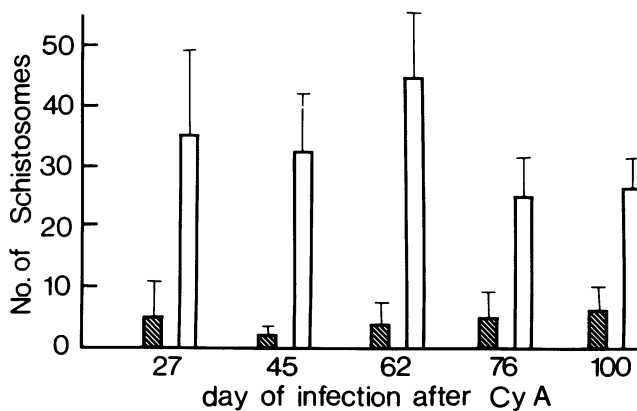

FIG. 6. Number of worms (mean ± standard deviation) recovered from CyA-treated and control C57BL/6 mice. Mice (8 to 10 per group) were infected with 60 cercariae at different times after CyA (▨) or solvent (▭) administration and perfused 35 days after infection.
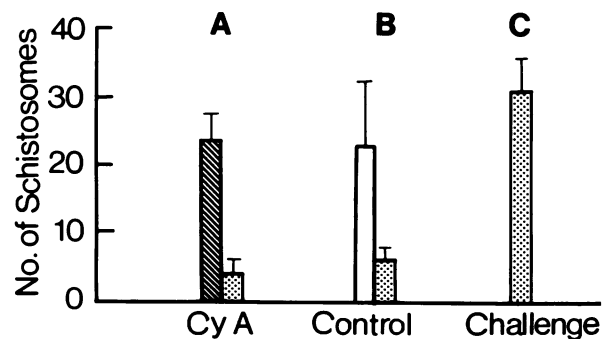

FIG. 7. C57BL/6 mice (10) in groups A and B were infected at day 0 and reinfected at day 42 with 60 and 100 cercariae, respectively. They received CyA (A) or solvent (B) from day 41 to day 45. Mice in group C were challenge controls. All of the mice were perfused at day 66. The number of worms recovered from first infection (▨ and ▭) and reinfection (▧) are shown (mean ± standard deviation).

and 21 days after challenge), the established adult schistosomes were not eliminated, whereas the challenge worms were eliminated (Fig. 7). When CyA (10 mg/kg of body weight per day) was injected from day 41 to day 66, 24 ± 2.9 mature and 3.4 ± 2.1 immature schistosomes, established from primary and secondary infection, respectively, were recovered from treated mice, as compared with 23.4 ± 8.3 and 5.2 ± 1.3 recovered from control mice.

Effect of CyA administration on schistosomiasis in rats. (i) Infection. In two different experiments, Fisher rats (three CyA-treated or untreated animals per group) were infected with 500 cercariae on day 0, and CyA (15 mg/kg of body weight per day) or solvent was injected from day −1 to day 3. Liver perfusion was performed at day 21, i.e., before spontaneous rejection of worms at day 28. Total protection was obtained in CyA-treated rats, in comparison with untreated rats (mean worm recovery, 84 ± 5.2 and 128 ± 3.5, respectively) in the two experiments.

(ii) Infection and reinfection. Fisher rats (six CyA-treated or untreated animals per group were infected on day 0 with 500 cercariae, and CyA (15 mg/kg of body weight per day) or solvent was injected from day −1 to day 3. From each group of six animals, three rats were perfused at day 21, whereas the three others were reinfected with 500 cercariae at day 45 and perfused at day 67, i.e., 22 days after challenge. Results of perfusion at day 21 showed almost total protection in CyA-treated rats (mean worm recovery, 0.5 ± 0.7) as compared with control rats (mean worm recovery, 101 ± 5). Total protection was observed when perfusion was performed on day 67 in infected and reinfected rats, as compared with control rats (mean worm recovery, 27.4 ± 9.6).

DISCUSSION

This study was initiated to define the controversial involvement of T-cell-dependent mechanisms in mouse resistance to reinfection by schistosome. It was expected that giving CyA, a well-known immunosuppressive agent, daily throughout the entire experiment would result in a worm burden during the primary infection identical to that obtained without giving any drug (Fig. 1). It was expected that if a T-cell-dependent mechanism was involved in resistance to reinfection, a challenge worm burden similar to that observed in the challenge control would result. Surprisingly, in such an experiment no worms were recovered from either primary or secondary infection. Similar results were shown with the rat model.

The protective effect of CyA was confirmed in infection experiments by injecting the drug either daily during the course of infection or for 5 days (day −1 to day 3) at the beginning of the infection. CyA injected once on day 0, day 1, day 2, or day 3 resulted in lower protection. This result indicates that the optimal level of CyA was not reached after one injection of the drug.

By injecting CyA only from day −1 to day 3 in the infection-reinfection model, a high degree of protection was obtained not only against the primary infection but also against the secondary infection, i.e., 42 days after the last injection of CyA. By using lung, liver, or total perfusion of the worms according to sequential timing, parasite elimination was shown to be predominantly an early event. The target of CyA thus seems to be the immature worms in the primary infection, as well as in the secondary infection. The long-term effect of CyA on protection was confirmed by experiments in which the drug was given 27, 45, 62, 76, or 100 days before infection. Praziquantel and Oxamniquine are

presently the drugs of choice in the treatment of schistosomiasis. They are active against both young and adult *S. mansoni* but do not have any preventive effect (1). The adult schistosomes were shown to be insensitive to CyA, since drug administration 41 days after infection did not affect the worm burden. This finding is in agreement with recent results of Nilsson et al. (13) but in disagreement with those of Bueding et al. (6), who showed a 21 to 30% reduction in the number of worms when CyA (25 mg/kg of body weight per day for 5 successive days) was administered 33 days after infection and 50 to 60 days before recovery of worms. It is possible that the differences in protocols could account for the discrepancies.

Because it has been shown that, in the dog, CyA is almost completely cleared within 96 h (2), and because CyA is known to act preferentially on T cells, we hypothesized that CyA provides protection by interfering with the immune system. This hypothesis was not confirmed by experiments using nude mice which were also protected by CyA administration. Since T cells are the selective targets of CyA (18), such results seem to eliminate a possible action of the drug via the immune system and to favor a direct effect on the schistosome. In addition, a high degree of protection was also obtained in other mouse strains with immune defects, such as CBA/N mice (whose B cells do not respond to TI-2 antigens, to low doses of TI-1 antigens, and, possibly, to some T-cell-dependent antigens) and P/N mice (which lack macrophage schistosomulicidal activity) (9).

CyA has also been shown to provoke a protective response against other helminth infections, such as filariasis (4), and some protozoan infections, such as malaria (12, 17) and toxoplasmosis (10; J. M. Hoffin, R. E. McCabe, and J. S. Remington, Fed. Proc. **43:**2069, 1984), but not against others, such as trypanosomiasis (11) and giardiasis (1a). Our data confirm and define the curative and preventive effect of CyA against schistosomiasis. It would be worthwhile to know the exact mechanism of action of this drug against these parasites. Other results with CyA and its nonimmunosuppressive analogs are in agreement with the preliminary data obtained with nude mice and indicate that an immune mechanism does not seem to be responsible for cure or prevention of schistosomiasis.

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