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Dosage, Timing, and Route of Administration of Cyclosporin A and Nonimmunosuppressive Derivatives of Dihydrocyclosporin A and Cyclosporin C against *Schistosoma mansoni* In Vivo and In Vitro

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The prophylactic and therapeutic effects of cyclosporin A (CsA) against percutaneous *Schistosoma mansoni* infection in MF1 mice were dose related and dependent on the temporal relationship between drug administration and infection. Antischistosomal activity, assessed by worm recovery from the host 6 weeks after infection, was most effective (complete worm elimination) when CsA was administered at the time of infection. Oral administration of CsA was less effective than subcutaneous injection, and no prophylactic activity was demonstrated by the former route. Derivatives of dihydrocyclosporin A and cyclosporin C, which have been reported to exert only poor immunosuppressive activity, exhibited efficacy against *S. mansoni* similar to that of CsA and were also less effective when given orally. Subcutaneous, but not oral CsA reduced cercarial skin penetration and transformation success; the derivative of dihydrocyclosporin A, however, was without effect. Moreover, CsA, but not the derivative of dihydrocyclosporin A, reduced the number of worms established after intraperitoneal injection of cercariae. These data provide further insight into the antischistosomal activity of cyclosporins, which appears to be distinct from their immunomodulatory properties, since parasite killing was retained both in immunologically disparate mice and with poorly immunosuppressive cyclosporin derivatives.

The antiparasite effects of cyclosporin A (CsA) against a variety of species including *Schistosoma mansoni*, *Plasmodium* sp., and *Dipetalonema viteae*, have been revealed in a number of recent publications (5–8, 10, 12–14). Work from this laboratory has demonstrated that the unexpected prophylactic activity of CsA against murine *S. mansoni* diminishes at 2 to 4 months preinfection (15; S. W. G. Smith, L. H. Chappell, A. W. Thomson, A. P. MacGowan, and J. G. Simpson, Int. Arch. Allergy Appl. Immunol., in press), while the therapeutic efficacy of the drug also declines with age of infection.

It is not understood whether the well-described immunomodulating activity of CsA is responsible, in any way, for the antischistosomal properties of the drug. Consequently, we have addressed this question through the use of poorly immunosuppressive derivatives of dihydrocyclosporin A (DHCsA-d) and cyclosporin C (CsC-d) (17), in vivo and in vitro. Also, we report on drug efficacy against *S. mansoni* when the drug is administered orally. A variety of mouse strains has been used in this study to ascertain whether there may be interstrain differences in the responsiveness of the host to cyclosporins.

MATERIALS AND METHODS

Parasite maintenance. *S. mansoni* (Wellcome strain) was maintained in laboratory-bred *Biomphalaria glabrata* and male MF1 mice. For certain experiments, male C57/BL10, CBA/Ca, and BALB/c mice were infected. Infection was by the abdominal ring method, whereby Sagatal (May & Baker Ltd., Dagenham, Essex, United Kingdom)-anesthetised animals received 100 freshly liberated cercariae of mixed sex. For one experiment, mice were infected by intraperitoneal (i.p.) injection of 100 cercariae in 0.2 ml of sterile artificial pond water; these animals were maintained on antibiotic added to the drinking water for 7 days postinfection.

Drug administration. CsA, DHCsA-d (B-5-49), and CsC-d (C-5-34) were a gift of J. F. Borel (Preclinical Research, Sandoz Ltd., Basel, Switzerland); they were administered to mice at the required dose either by subcutaneous (s.c.) injection under the dorsal skin immediately posterior to the neck or by oral catheter into the stomach. In all cases, drug was delivered in 0.1 ml of vehicle (olive oil-absolute ethanol, 9:1, vol/vol). Drug treatment regimes were timed to coincide with infection (day 0) or at prescribed times pre- or postinfection. All control animals received 0.1 ml of vehicle by the appropriate route.

Collection of parasites. At 6 weeks postinfection, mice were killed by cervical dislocation and schistosomes were removed by aortic perfusion with citrate saline (4°C). The sex of the worms thus collected was determined, they were counted, and their dry weights were recorded on a Mettler M3 microbalance (accurate to 1 μg). For all mice used, the body weights and fresh weights of liver and spleen were determined.

In vitro skin penetration studies. The abdominal skin of drug-treated mice (and vehicle-treated controls) was shaved and excised. After mechanical removal of the majority of the subcutaneous fat, each skin was placed in a glass penetration tube (9) in the lower half of which was medium 199 supplemented with 10% newborn calf serum and 1% antibiotic/antimycotic (GIBCO, Paisley, Scotland). A cercarial suspension containing 1,000 to 2,000 cercariae in 2 ml of artificial pond water was added to the upper half of the apparatus; both halves were held together with a stainless-steel (Quickfit) clip. The apparatus was maintained in a static

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TABLE 1. Effects of therapeutic or prophylactic s.c. CsA treatment on *S. mansoni* in vivo[a]

Day central to treatment regime[b]	Mean % reduction in recovery of adult *S. mansoni*		Mean % reduction in dry wt/worm	
	Male worms	Female worms	Male worms	Female worms
0				
3 × 10 mg kg^{-1}	53.0**	15.8	26.9	0
3 × 20 mg kg^{-1}	99.9**	100	61.5***	
3 × 30 mg kg^{-1}	100	100		
3 × 50 mg kg^{-1}	100	100		
−9				
3 × 10 mg kg^{-1}	0	0	0	33.3**
3 × 20 mg kg^{-1}	70.3**	38.5	63.6**	44.4**
3 × 30 mg kg^{-1}	62.2**	84.6**	40.9*	22.2**
3 × 50 mg kg^{-1}	100	100		
−13				
1 × 50 mg kg^{-1}	0.8	0	0	0
2 × 50 mg kg^{-1}	74.2***	85.0***	45.7***	0
3 × 50 mg kg^{-1}	79.4***	82.0**	49.6**	91.4**
1 × 250 mg kg^{-1}	67.5***	79.0***	51.7***	25.0
−57				
1 × 50 mg kg^{-1}	0	0	0	0
2 × 50 mg kg^{-1}	0	0	0	0
3 × 50 mg kg^{-1}	23.9	42.1	0	0
1 × 250 mg kg^{-1}	0	0	17.1	18.2

[a] *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (comparison of mean data on worm numbers by Student's t test against controls). n = 5 or 6 mice per group.

[b] Drug treatment regimes: day 0, administration of CsA on days −1, 0, and +1 relative to infection on day 0; day −9, administration of CsA on days −10, −9, and −8 relative to infection on day 0 (i.e., 7 clear days between last drug dose and infection); day −13, administration of CsA on days −15, −14, and −13, −13 and −12, or −12 (depending on group) relative to infection on day 0; day −57, administration of CsA relative to infection on day 0. 3 × 10 mg kg^{-1} = three 10-mg kg^{-1} doses, etc.

37°C water bath in an ambient environment of 26°C. Cercariae were allowed to penetrate the excised mouse skins for 4 h, after which time the numbers of schistosomula that had accumulated in the lower half of the apparatus were counted. The area of skin available for cercarial penetration was 1.54 cm^2. Skins were fixed in 10% neutral buffered Formalin for routine histology.

Skin histology. A section of skin across a diameter of the area to which cercariae had been applied was processed to paraffin wax, sectioned at 5 μm, and stained with hematoxylin and eosin. The number of schistosomula per section was determined by microscopic examination.

RESULTS

CsA dose ranges s.c. A range of doses of CsA was administered to MF1 mice either coincident with infection (day 0; "therapeutic") or over one of three periods prior to infection, and worms were recovered at 6 weeks postinfection (Table 1). Complete elimination of schistosomes was achieved by therapeutic CsA at three doses of 20 mg kg^{-1} or greater each, while only partial killing was observed with three doses of 10 mg kg^{-1} each. The lower doses tended to affect male worms preferentially, in terms of both numbers recovered and dry weight. The higher drug doses used (≥20 mg kg^{-1}) significantly reduced the splenomegaly associated with infection, but in only one case (three doses of 20 mg kg^{-1} each; around day 0) reduced liver weight (data not shown).

The antischistosomal effects of CsA when administered prophylactically diminished with decreasing drug dose and with time elapsed before infection. In only two of these treatments (two doses of 50 and one dose of 250 mg kg^{-1} each; day −13) was a significant diminution in splenomegaly observed, and no significant effect on liver weights was recorded. It is noteworthy that a single administration of 250 mg of CsA kg^{-1} around 13 days before infection was as effective as dual or triple doses of 50 mg kg^{-1}, but by day −57 only a regime of three 50-mg kg^{-1} doses exerted any antischistosomal activity. In contrast with therapeutic treatment, there was no clear evidence of sexual bias with prophylactic CsA administration.

Oral CsA dose ranges. CsA courses were administered orally to MF1 mice around either day 0 or day −9 relative to infection. It is apparent from the data in Table 2 that the effects of drug against worms were considerably reduced when CsA was given orally. Moreover, little or no prophylaxis was evidenced with treatment around 9 days before infection. Also, oral drug failed to consistently reduce hepatosplenomegaly (data not shown), and no evidence of sex selectivity was obtained. The highest dose used (five doses of 150 mg kg^{-1} each) only reduced the numbers of male worms by 42% and was less effective against females. However, despite the lack of effect on worm recovery, oral CsA reduced the size of worms recovered at perfusion, but again little sexual preference in drug action was recorded. It is evident (Table 2) that oral administration of CsA yields highly variable results in terms of reduction in worm recovery, as shown by the replicate (day 0) data, which is further reproduced in Table 3. In this regard, oral drug treatment is less satisfactory than s.c. administration, an occurrence for which there is no obvious explanation.

Treatment s.c. and orally with DHCsA-d and CsC-d. The two available cyclosporin derivatives DHCsA-d and CsC-d

TABLE 2. Effects of therapeutic or prophylactic oral CsA treatment on *S. mansoni* in vivo[a]

Day central to treatment regime[b]	Mean % reduction in recovery of adult *S. mansoni*		Mean % reduction in dry wt/worm	
	Male worms	Female worms	Male worms	Female worms
0 (expt 1)				
3 × 25 mg kg^{-1}	68.6***	51.9*	30.9	12.5
3 × 50 mg kg^{-1}	59.0**	61.5**	23.6*	25.0
3 × 75 mg kg^{-1}	63.8**	76.9***	14.5	0
2 × 125 mg kg^{-1}	38.1*	57.7**	16.4	12.5
1 × 250 mg kg^{-1}	51.5**	53.8*	0	0
0 (expt 2)				
5 × 25 mg kg^{-1}	15.8	22.4	0	26.7**
5 × 50 mg kg^{-1}	21.0	24.1	10.9	20.0*
5 × 75 mg kg^{-1}	47.4**	44.8*	32.6**	20.0*
5 × 150 mg kg^{-1}	42.3*	22.9	31.2**	29.4**
−9 (expt 2)				
5 × 25 mg kg^{-1}	0	3.9	12.0*	0
5 × 50 mg kg^{-1}	0	0	0	0
5 × 75 mg kg^{-1}	0	26.7	12.0	7.1

[a] n = 5 or 6 mice per group. *, **, ***, Statistical differences as for Table 1 (footnote *a*).

[b] Drug treatment regimes: day 0, CsA administered either 1 (three times [3×]) or 2 (five times [5×]) days on either side of and on the day of infection (day 0), the day before and on the day of infection (two times [2×]), or on the day of infection (once [1×]); treatment around day −9 allowed 6 clear days between the last treatment and infection.

were compared with the parent compound in s.c. and oral trials, using a regime of five doses of 50 mg kg^{-1} each. Both derivatives exhibited efficacy against *S. mansoni* similar to that with CsA when given either at the time of infection (around day 0) or prophylactically (around day −9) (Table 3). The observed reduction in liver and spleen size was variable (data not shown), although DHCsA-d consistently reduced splenomegaly. Oral treatment was once again convincingly less effective than s.c. delivery.

In vitro cercarial penetration and transformation. Skin penetration and transformation to schistosomula were used to assess the role of host skin in cyclosporin-related attrition of schistosomes. Drug regimes of three and four doses of 50 mg of CsA kg^{-1} each significantly reduced the transformation success of mixed-sex and male cercariae when compared with controls; this effect diminished when skins were removed 7 days after treatment compared with 1 day post-treatment (Table 4). DHCsA-d did not reduce cercarial transformation in either MF1 or CBA/Ca mice and, furthermore, oral CsA treatment was without effect.

Histological examination of these skins indicated that, despite reduction in transformation success attributable to drug treatment, there were no apparent accumulations of parasite larvae within the skin, suggesting that perhaps fewer cercariae successfully entered the treated skin.

Establishment of *S. mansoni* from i.p. injected cercariae in mice treated with CsA or DHCsA-d. To assess whether the skin forms a site of parasite attrition in cyclosporin-treated animals, it was felt essential to examine the establishment of parasites in drug-treated mice in which the skin was bypassed by the cercariae; thus, mice were infected by i.p. injection coincident with administration of cyclosporin. This method of infection leads to poor establishment of adult schistosomes compared with the conventional skin penetration method. CsA reduced the numbers of parasites established at 6 weeks postinfection by a level similar to its efficacy with normal skin-penetrating cercariae (Table 5). Following CsA treatment, male worms were significantly smaller in both mouse strains, but female worms from MF1 mice remained unaltered in weight. By contrast with CsA, DHCsA-d did not alter the numbers of worms becoming established after i.p. injection of cercariae to the extent achieved by CsA and also was without much effect on worm size at perfusion (Table 5).

Antiparasite efficacy of CsA in different mouse strains. CsA treatment (three doses of 50 mg kg^{-1} each) coincident with infection of male CBA/Ca, C57/BL10, and BALB/c mice compared closely in its effect on worm recovery to the results obtained with MF1 mice. Greater than 98% reduction in recovery of male and 100% reduction in recovery of female worms were recorded in each strain. In no case was the host liver size affected, but splenomegaly was significantly reduced in all strains.

Susceptibility of adult *S. mansoni* to high-dose CsA and DHCsA-d. It is controversial whether adult schistosomes are susceptible to CsA treatment; our data (Smith et al., in press) show that a 30% reduction in worm recovery can be achieved by s.c. administration of five doses of 50 mg of CsA kg^{-1} each to mice carrying a 7-week-old infection. To examine this further and to investigate the hypothesis that cyclosporins act primarily against juvenile schistosomes, MF1 mice with patent (7-week-old) infections were given either five doses of 50 or 150 mg of s.c. CsA or DHCsA-d kg^{-1} each and were autopsied 14 days later (Table 6). High doses of CsA were less effective against schistosome survival than lower doses, which also significantly reduced hepatosplenomegaly. Also, high-dose CsA had no effect on worm size at recovery, whereas DHCsA-d significantly reduced the weight of female worms. It is interesting to record the virtual absence of effect of DHCsA-d on worm recovery when given to mice with 7-week-old infections (Table 6) compared with its potent activity when administered at the time of infection (Table 3). Three of the four treatments used in this experiment reduced the size of female worms selectively.

TABLE 3. Effects of s.c. or oral treatment regimes of CsA, DHCsA-d, or CsC-d on *S. mansoni* in vivo[a]

Day central to treatment regime[b]	Mean % reduction in recovery of adult *S. mansoni*		Mean % reduction in dry wt/worm	
	Male worms	Female worms	Male worms	Female worms
0 (s.c.)				
CsA	100	100		
DHCsA-d	98.5***	100		
CsC-d	97.8***	100		
−9 (s.c.)				
CsA	99.3***	100	65.8	
DHCsA-d	51.9	37.5	39.5	45.4
CsC-d	87.8***	90.2***	68.4	45.4
0 (oral)				
CsA	57.0*	59.3*	22.9*	46.7***
DHCsA-d	50.4*	45.9*	0	0
CsC-d	48.3*	44.8*	12.5*	6.7
−9 (oral)				
CsA	14.1	42.3	19.6*	11.1
DHCsA-d	16.7	8.2	10.7	11.1
CsC-d	1.5	21.9	14.3	5.5

[a] n = 5 or 6 mice per group. *, ***, Statistical differences as for Table 1 (footnote *a*).

[b] Drug treatment regimes: day 0, five daily doses of 50 mg kg^{-1} each were administered on days −2, −1, 0, 1, and 2, relative to infection on day 0; day −9, five daily doses of 50 mg kg^{-1} each were administered on days −11, −10, −9, −8, and −7, relative to infection on day 0.

DISCUSSION

The mode of action of cyclosporin against any parasite remains unexplained. It is also evident that cyclosporin may act against different parasites in different ways and, indeed, may be without effect on certain species, e.g., *Giardia muris* and *Trypanosoma cruzi* (4, 11). Three alternative models can be proposed to account for the antischistosomal activity of this family of fungal metabolites: (i) direct action of either parent compound or metabolite(s) on the parasite; (ii) immunomodulation by parent drug or metabolite(s); (iii) activation by drug of a population of cells with potent antischistosomal properties.

Data presented here and elsewhere (8; Smith et al., in press) do not support the notion that native CsA exerts a direct effect on schistosomes. CsA has no effect on schistosomula or adult *S. mansoni* in vitro, nor does it inhibit isolated parasite hemoglobinase directly. Also, the present observations that (i) poorly immunosuppressive cyclosporin derivatives possess activity similar to that of CsA against schistosomes and (ii) the antischistosomal properties of these cyclosporins are retained in genetically disparate mice (C57/BC10, CBA/Ca, and BALB/c), as well as in athymic animals (5), argue compellingly that the antiparasite activi-

TABLE 4. Reduction of penetration/transformation success of *S. mansoni* cercariae via excised skin in vitro following cyclosporin treatment of mice[a]

Mouse strain	Treatment regime (mg kg^{-1})[b]	Drug	Route of drug administration	Mean % reduction in transformation to schistosomula	Mean no. of schistosomula/skin section ($x \pm$ SD)	
					Control	Treated
MF1	4× 50	CsA[c]	s.c.	51.3*	19.8 ± 8.5	16.0 ± 9.3
MF1	4× 50	CsA[d]	s.c.	38.6*	22.0 ± 13.3	21.0 ± 10.1
MF1	3× 50	CsA[e]	s.c.	41.2*	13.4 ± 7.1	25.4 ± 14.4
MF1	3× 50	DHCsA-d[c]	s.c.	0	34.8 ± 14.9	34.3 ± 21.1
MF1	4× 75	CsA[c]	Oral	3.2	43.8 ± 6.4	39.5 ± 12.5
MF1	4× 150	CsA[c]	Oral	15.6	43.8 ± 6.4	50.2 ± 17.7
CBA/Ca	4× 50	CsA[c]	s.c.	73.4*	44.0 ± 1.0	48.3 ± 12.3
CBA/Ca	4× 50	DHCsA-d[c]	s.c.	0	44.0 ± 1.0	40.0 ± 17.7

[a] $P < 0.05$; comparison of mean numbers of schistosomula obtained against controls, which received vehicle only. n = 4 or 5 skin preparations per group.
[b] 4× 50 mg kg^{-1} = four 50-mg kg^{-1} doses, etc.
[c] Skins excised 1 day post drug treatment; mixed-sex cercariae.
[d] Skins excised 7 days post drug treatment; mixed-sex cercariae.
[e] Skins excised 1 day post drug treatment; male cercariae.

ties are not mediated via immunomodulation but are indeed quite distinct processes. Nevertheless, augmentation of delayed hypersensitivity responses of rodents to certain nonparasite antigens by CsA pretreatment has been described (1, 15a), in which event it appears that a population of suppressor cells may fail to become activated. The latter, immunopotentiating effect of CsA is dependent upon both timing and dose of drug administration: similarities to the antischistosomal properties of CsA in both cases may imply that augmentation of the immune response could, conceivably, be related to parasite killing, but this clearly requires further investigation.

The inferior efficacy of oral compared with s.c. cyclosporin may be explained by comparatively poor absorption of drug in the mouse intestine or by significant levels of hydrolysis in the upper alimentary tract. The oral route, however, is preferred for the clinical administration of CsA in the management of organ transplantation and the tissue distribution of CsA is similar in mice receiving s.c. drug and humans receiving drug by the oral route (1). After oral administration of CsA, its bioavailability is 20 to 50% and drug is extensively distributed in human extravascular tissues (16). Thus, the reduced antiparasitic effects of oral cyclosporin are difficult to explain. Since oral treatment is sufficient to produce significant immunomodulation in humans and other animals, our data may be regarded as additional, circumstantial evidence that cyclosporin acts against schistosomes by mechanisms divorced from immune modulation. As high doses of oral CsA (five does of 150 mg kg^{-1} each) are only partially schistosomicidal and such levels are likely to be toxic to the host, particularly if treatment were to be extended (3), it is possible that oral administration of the cyclosporins examined to date will be of restricted value in the control of human schistosomiasis.

Evidence currently available strongly suggests that cyclosporin is most active against juvenile schistosomes (5, 8, 15). Nevertheless, our data (Table 6) (Smith et al., in press) clearly demonstrate that both lung stage and adult worms are partially susceptible to the drug. This implies that parasite attrition can occur at a variety of sites within the host but that the skin may be the primary site of parasite death. This proposition is supported, to some extent, by the in vitro skin penetration studies we have reported. The prediction that the skin represents the major locus of schistosome death was, however, not upheld by our observations on infections established from i.p. injection of cercariae to bypass the skin and associated dermal tissues (Table 5). In this experiment, CsA clearly retained its antischistosomal properties, whereas DHCsA-d exerted differential effects, depending on mouse strain. Clearly, further useful information will be

TABLE 5. Effect of CsA or DHCsA-d on establishment of i.p. injected cercariae of *S. mansoni*[a]

Mouse strain	Drug treatment regime	Mean no. of *S. mansoni* recovered ($x \pm$ SD)[b]		Mean dry wt/worm (μg)	
		Male worms	Female worms	Male worms	Female worms
MF1	Control[c]	6.7 ± 2.1	7.7 ± 5.6	23 ± 12	11 ± 4
	CsA[d]	1.0 ± 1.0**	0.2 ± 0.4**	5 ± 3**	11 ± 0
	DHCsA-d[d]	10.3 ± 8.9	2.0 ± 2.9	19 ± 24	22 ± 0**
CBA/Ca	Control[c]	15.0 ± 11.5	17.2 ± 13.0	24 ± 7	10 ± 2
	CsA[d]	1.4 ± 2.3**	0	3 ± 3	
	DHCsA-d[d]	6.0 ± 0.8*	3.0 ± 1.4**	16 ± 4	9 ± 1

[a] n = 5 mice per group. *, **, Statistical differences as for Table 1 (footnote *a*).
[b] Worms were 5.5 weeks old at perfusion.
[c] Controls were given five daily injections of vehicle.
[d] Drug administered at five doses of 50 mg kg^{-1} each s.c. 2 days before, on the day of, and for 2 days after infection.

TABLE 6. Effects of CsA and DHCsA-d on adult *S. mansoni* infections[a]

Drug treatment regime[b]	Mean % reduction in worm recovery		Mean dry wt/worm (μg), $x \pm$ SD	
	Male worms	Female worms	Male worms	Female worms
Control			93.0 ± 6.0	38.0 ± 5.0
CsA				
5× 50 mg kg^{-1}	28.4	43.0*	87.0 ± 8.0	12.0 ± 2.0*
5× 150 mg kg^{-1}	5.7	32.4	86.0 ± 12.0	42.0 ± 47.0
DHCsA-d				
5× 50 mg kg^{-1}	0	7.2	104.0 ± 7.0	12.0 ± 1.0*
5× 150 mg kg^{-1}	0	17.5	96.0 ± 5.0	13.0 ± 2.0*

[a] n = 5 mice per group. *, Statistical differences as for Table 1 (footnote *a*).
[b] Drug treatment regime: five (5×) s.c. daily doses commencing when infection reached 7 weeks. Mice were killed 14 days after final drug treatment (i.e., 10-week-old worms at perfusion).

obtained by an examination of the profile of parasite attrition in vivo and in vitro, using radiolabeled cercariae.

The novel information contained in this paper underlines the unique and exciting properties of cyclosporins as antischistosomal agents. Their future development will depend upon an understanding of mode of action, site of parasite death, and, in particular, the mechanisms responsible for the prophylactic properties of these drugs. It is disappointing that oral administration of cyclosporin results in reduced parasite kills but, with extended knowledge, perhaps novel derivatives can be developed that retain potent activity against parasites when administered orally. Regardless of route of administration, CsA is metabolized slowly but extensively in all mammals examined, and of the 17 metabolites that have been found, at least 9 have been isolated and identified (18). It may therefore be no simple task to identify which, if any, of these metabolites is responsible for antiparasite activity. While there are no published data on the serum or tissue half-lives of the derivatives used in this study, it has been reported that CsA can remain in tissues for a considerable time (i.e., weeks) after drug withdrawal (2, 17). The latter observation could account for the remarkable prophylactic effects we have observed. Without question, considerable effort is required to identify the candidate model for the action of cyclosporins and the data presently available provide only modest clues to this intriguing problem.

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