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Cyclosporin A Inhibits the Growth of *Cryptococcus neoformans* in a Murine Model

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***Cryptococcus neoformans* is a frequent opportunistic infectious agent in patients with decreased T-lymphocyte-mediated immune function, including those with acquired immune deficiency syndrome. Cyclosporin A (CsA), a potent inhibitor of T-lymphocyte function, was administered subcutaneously to mice to study the pathogenesis of *C. neoformans* infections in the setting of impaired T-cell function. Surprisingly, survival was prolonged indefinitely in animals that received immunosuppressive doses of CsA following either intratracheal or intravenous inoculations of *C. neoformans*. Furthermore, following intratracheal inoculation, mice treated with CsA cleared *C. neoformans* from their lungs more rapidly than did control mice. CsA directly inhibited the growth of *C. neoformans* when it was added to cultures in vitro at concentrations comparable to the blood levels achieved in experimental mice. Thus, CsA inhibited both in vitro and in vivo growth of *C. neoformans*. While these results must be extended to studies in humans, these data suggest that patients who now receive CsA-immunosuppressive therapy may be fortuitously protected against infections with *C. neoformans*. Furthermore, research into cyclosporin derivatives may yield compounds with less immunosuppressive properties and enhanced antifungal activity.**

Cryptococcus neoformans is a frequent opportunistic pathogen in humans, particularly in patients with defects in cell-mediated immunity (10, 14). *C. neoformans* is an important pathogen in patients with acquired immune deficiency syndrome, in whom there is a profound deficiency of T helper lymphocytes (14). Although the mechanism of protection against *C. neoformans* in humans is incompletely understood, the increased incidence in patients with defects in cell-mediated immunity suggests that functional T cells are important in protection (22). The present study was performed to develop a model of cryptococcal infection in the setting of impaired T-cell function.

Cyclosporin A (CsA) is a cyclic endecapeptide originally derived as a crude extract of two strains of Fungi Imperfecti, *Cylindrocarpum lucidum* Booth and *Tolypocladium inflatum* Gams. Although initially discovered while attempting to identify new antifungal agents, CsA is now used widely as a potent immunosuppressive agent, particularly in human allograft transplantation (9).

The mechanism of immunosuppression induced by CsA has been extensively studied in animals and humans. CsA-mediated T-cell immunosuppression is believed to be due to the decreased release of interleukin 2 from stimulated T lymphocytes, resulting in a failure to develop a specific immune response (8, 12). B lymphocytes are affected only secondary to failure of the T helper cells (4), and the effector activity of macrophages has been shown to be normal (27). We treated mice with daily injections of CsA and inoculated them with pathogenic encapsulated *C. neoformans* to study the pathogenesis of *C. neoformans* infections in a murine model of T-cell dysfunction. To our surprise, CsA treatment prolonged survival indefinitely. Furthermore, pulmonary clearance of *C. neoformans* was enhanced in CsA-treated mice, and the enhanced clearance in vivo correlated with a direct effect of CsA on *C. neoformans* growth in vitro.

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MATERIALS AND METHODS

Animals. Adult male C57BL/6 mice were obtained from Jackson Laboratory (Bar Harbor, Maine) at 6 to 8 weeks of age. They were housed in a sterile filter hood and monitored for mycoplasma and Sendai virus infections. All animals received mouse chow and sterile water ad libitum.

CsA. CsA (Sandimmune IV) was donated by Sandoz Inc. (East Hanover, N.J.). CsA was diluted with normal saline (final concentration, 2.5 or 5 mg/ml). A control solution was prepared with the carrier for CsA, 3.25 ml of Cremaphor-EL (Cr-E; Sigma Chemical Co., St. Louis, Mo.), plus 1.6 ml of ethanol, and diluted to correspond to the carrier concentration in CsA with normal saline for subcutaneous administration.

CsA assay. Whole blood (0.5 to 1.0 ml) was obtained from the retroorbital plexus of mice in heparinized tubes (75 μl of a 1:10,000 solution). CsA was extracted and quantified by high-performance liquid chromatography (18). Briefly, 0.25 ml of whole mouse blood was combined with 0.75 ml of pooled human blood. To each tube, 2 ml of internal standard (cyclosporin D; 250 ng/ml in acetyl nitrite-methanol [9:1]; Sandoz Inc.) was added. Specimens were then vortexed and centrifuged, and the supernatant was combined with 1 ml of H_2O. Samples were then bound to a 6-ml, reverse-phase column (C_{18} column; Analytichem, Harbor City, Calif.) and washed with 70% methanol–1% acetone in hexane. Cyclosporin was eluted from the column with ethyl acetate in isopropanol (3:1). Specimens were further purified on a 3-ml silica column (SI column; Analytichem), dried, and reconstituted with acetyl nitrite and methanol in H_2O (1:1:3). Samples were run in an isocratic mobile phase (40% acetyl nitrite, 30% methanol, 30% H_2O) over a reverse-phase column (LC-1; Pelco, Bellefonte, Pa.) and analyzed for optical density at 214 nm by using a high-performance liquid chromatograph (Waters Associates, Inc., Millford, Mass.).

A standard curve was constructed with various known concentrations of CsA. The unknown concentration of CsA was determined from the ratio of CsA to cyclosporin D in each sample by comparing it with the standard curve.

C. neoformans. *C. neoformans* 145A (serotype A) was generously donated by Judith E. Domer, Tulane University Medical Center, New Orleans, La. *C. neoformans* ATCC 36556 was obtained from the American Type Culture Collection, Rockville, Md. *C. neoformans* H99 was generously donated by John Perfect, Duke University, Durham, N.C. Strains were maintained on Sabouraud agar slants (Difco Laboratories, Detroit, Mich.) and passed to fresh slants monthly. For experiments, organisms were transferred from slants to 1% neopeptone–2% glucose broth (Difco) and placed onto an orbital shaker at 35°C (Lab Line Instruments, Melrose Park, Ill.) for 48 h. Organisms in broth were washed three times by centrifuging at 400 × *g* for 10 min and suspending them in normal saline. Organisms were initially counted with a hemacytometer and suspended in normal saline for inoculation into mice, or in broth as described below for in vitro sensitivity. Hemacytometer counts were verified by plating serial dilutions of the inoculum onto culture plates containing Sabouraud agar.

Protocol for evaluating the effect of CsA on the growth of *C. neoformans* in vitro. Broth (5 ml) containing 5×10^3 organisms per ml was placed in sterile, 50-ml conical tubes. CsA was added at concentrations of 1.0 and 0.1 μg/ml. Cr-E was added to a separate tube at a concentration equivalent to the highest concentration of CsA. Nothing was added to one tube to serve as a control. Culture tubes were incubated for 48 h as described above. The broth was vigorously vortexed, serial 10-fold dilutions of the broth were plated onto Sabouraud agar (Remel, Lenexa, Kans.) and incubated for 48 h, and the CFU/ml was enumerated. Percent inhibition was calculated as follows: percent inhibition = [(CFU in control − CFU in CsA or Cr-E)/CFU in control] × 100.

CsA administration. Mice received CsA by the subcutaneous (s.c.) route in various doses (20, 50, and 75 mg/kg per day). CsA was given daily, beginning on the day before inoculation with *C. neoformans*. Control mice received an equivalent amount of Cr-E on the same schedule.

***C. neoformans* administration.** *C. neoformans* was given either intravenously (i.v.) or intratracheally (i.t.). For i.v. inoculation, 10^3 organisms were suspended in normal saline (0.5 ml) and injected in a lateral tail vein. *C. neoformans* (10^4 to 10^6 CFU) was administered by the i.t. route as described previously (19). Briefly, animals were anesthetized with pentobarbital. The trachea was exposed, and the animal was intubated with a blunt-tipped, 20-gauge steel needle. A plastic cannula (PE-10 tubing; Clay Adams, Parsippany, N.J.) was inserted through the steel needle 2.8 cm from the teeth into the trachea. A 5-μl slurry containing *C. neoformans* was deposited by using 100 μl of air. Immediately after inoculation, two or three mice from each treatment group were sacrificed. The lungs were harvested, and the number of CFU was determined as described above. These CFU were considered the deposition by i.t. inoculation. The difference in CFU at deposition between the two treatment groups was not significantly different and are reported together.

Survival and clearance studies. In survival studies, animals were observed twice daily and sacrificed if moribund, and the number of days after inoculation was recorded. CFU was evaluated in moribund animals that were sacrificed. In clearance studies, five mice from both experimental and control groups were sacrificed at various times. Lung, spleen, kidney, liver, and brain were removed; and CFU was determined. Organs were placed in sterile plastic bags, 2 ml of H_2O was added, and then the organs were blended (Stomacher Lab Blender 80; Tekmar Co., Cincinnati, Ohio). In addition, lungs were ground with a Ten Broeck tissue grinder (Corning Glass Works, Corning, N.Y.). Serial 10-fold dilutions of each organ were performed and plated on Sabouraud agar (Difco) in duplicate, and the number of CFU was counted 48 h later.

Mitogen stimulation. Mice were given 75, 50, 20, or 0 (control mice) mg of CsA per kg per day for 7 days. They were then sacrificed, the spleens were removed, and a single-cell suspension was made by passage through a stainless steel mesh screen. Culture media consisted of RPMI 1640 medium containing 1 mM pyruvate, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 IU of penicillin per ml, 100 μg of streptomycin per ml, and 5% fetal bovine serum. Splenocytes (3×10^5) were suspended in 200 μl of medium in round-bottom, 96-well microtiter culture plates, and concanavalin A (2 μg/ml) was added. The cells were incubated for 48 h, pulsed with [^{3}H]thymidine, and harvested 18 h later.

Statistics. Values are expressed as mean ± standard error of the mean. Probability was calculated by unpaired two-tailed Student's *t* test statistics.

RESULTS

CsA levels in blood. CsA (Sandimmune IV) has not previously been administered s.c. in mice. Therefore, CsA levels in blood were measured in mice after inoculation via this route. Mice received 20, 50, and 75 mg/kg per day s.c. for greater than 7 days. Levels in blood 24 h after the last dose were 0.30 ± 0.03, 1.50 ± 0.10, and 2.75 ± 0.85 μg/ml, respectively.

Effect of CsA on survival of mice receiving *C. neoformans* i.v. The effect of CsA on survival following i.v. inoculation of *C. neoformans* was determined. Ten animals received CsA (75 mg/kg per day) and 10 received control solution beginning on the day before inoculation of the animal with *C. neoformans* 145A (10^3 CFU) in the lateral tail vein. The first animal in the control group died on day 21, and the mean survival time was 28.3 ± 1.2 days (Fig. 1). All animals in the control group who received only the carrier for CsA were dead 34 days after inoculation. Similar results were obtained for untreated animals in preliminary experiments (data not shown). All of the animals treated with CsA survived until the experiment was terminated on day 45 (11 days after the last death in the control group). At the completion of the experiment, animals that had received CsA had 1,000-fold less CFU in all organs (Table 1) than in animals in the control group.

Effect of CsA on in vitro growth of *C. neoformans*. It was possible that CsA directly inhibited the growth of *C. neoformans*. Furthermore, it was possible that growth inhibition occurred with additional strains of *C. neoformans*. Therefore, the effect of CsA on the in vitro growth of several strains of *C. neoformans* was evaluated. *C. neoformans* was cultured in neopeptone broth with CsA at concentrations of 0.1 and 1.0 μg/ml. Growth of *C. neoformans* in broth with Cr-E at a concentration equal to that present in the preparation of 1.0 μg of CsA per ml was also studied. The pH of broth containing CsA was 6.6, and the pH of broth containing Cr-E was 6.7. The pH of broth without additives was 6.2. Growth in broth without any additives served as a control. Partial inhibition was observed with strains 145A, ATCC

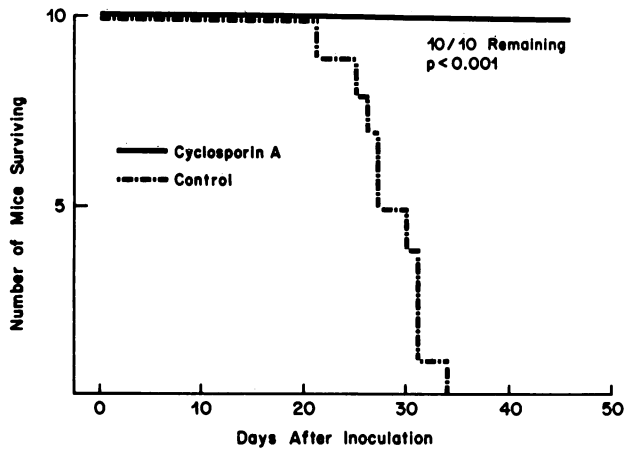

FIG. 1. Effect of CsA on survival of mice after i.v. inoculation with *C. neoformans* 145A (10^3 CFU). Mice (n = 10 per group) received 75 mg of CsA per kg per day or a control solution containing Cr-E, equivalent to 75 mg/kg per day, beginning on the day before inoculation ($P < 0.001$).

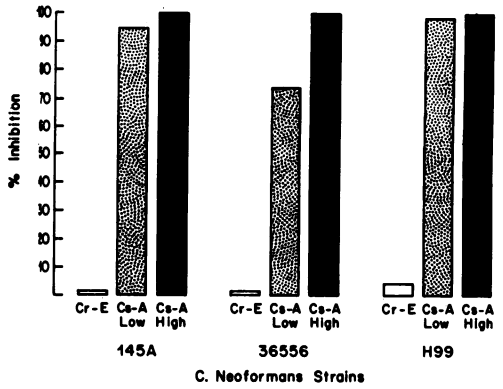

FIG. 2. Effect of CsA on in vitro growth of *C. neoformans* 145A, ATCC 36556, and H99 in the presence of Cr-E (□), low CsA (0.1 μg/ml; ▨), or high CsA (1.0 μg/ml; ■). Values are expressed as percent inhibition (see text).

36556, and H99 in broth containing CsA (0.1 μg/ml) after 48 h (Fig. 2). However, inhibition was complete (100%) with all strains at a concentration of 1.0 μg/ml. Furthermore, after prolonged incubation of broth containing CsA at a concentration of 1.0 μg/ml, no cloudiness was noted, indicating that there had been no growth. The growth in Cr-E and control media was equivalent. Similar results were observed with yeast nitrogen base medium (data not shown).

Effect of CsA on clearance in mice receiving *C. neoformans* i.t. It is likely that *C. neoformans* is acquired by the pulmonary route in humans. Therefore, the effect of CsA on lung clearance following i.t. inoculation of *C. neoformans* was examined. Animals received CsA (50 mg/kg per day) or a control solution beginning on the day before inoculation with *C. neoformans* 145A (10^4 CFU). The CFU in the lungs at the time of deposition was equivalent in both treatment groups. At 24 h there was a statistically significant difference in the CFU between the group receiving CsA and that receiving the control solution (Fig. 3). The CFU in the lungs of mice receiving CsA continued to decrease over 14 days. In contrast, in the control group, fungal replication continued, so that by 14 days there was a 10,000-fold difference in CFU between the group that received CsA and the group that received the control solution. Furthermore, 14 days after inoculation, five of five mice in the control group had dissemination of *C. neoformans* to the liver (70 ± 6 CFU), two of five had involvement of the spleen (115 ± 10 CFU) and the kidney (105 ± 9 CFU), and one had dissemination to the brain (20 CFU). In contrast, all mice that received CsA had sterile cultures of liver, spleen, kidney, and brain.

The effect on lung clearance of mice receiving CsA was further evaluated by using (i) a lower dose of CsA and (ii) two additional strains of *C. neoformans*. Animals received CsA (20 mg/kg per day) or the control solution beginning 1 day before inoculation with *C. neoformans*. As indicated above, trough levels with this dose were comparable to those present in patients on immunosuppressive protocols. Mice were inoculated with one of the following three strains of *C. neoformans*: 145A, ATCC 36556, H99. Four days after inoculation with *C. neoformans*, the CFU in the lungs of all mice receiving CsA decreased from the number of organisms deposited initially. In contrast, in the control groups, fungal replication continued, so that there was at least a ninefold increase in CFU between control mice and mice receiving CsA for all three strains of *C. neoformans* tested (Table 2).

TABLE 1. Effect of CsA on infection by *C. neoformans*[a]

Organ	CFU ($\log_{10}$) organ in mice at death[b]	
	Control	CsA Treated
Lung	6.65 ± 0.13	1.42 ± 0.34[c]
Spleen	5.96 ± 0.38	2.40 ± 0.42[c]
Kidney	5.66 ± 0.13	0.96 ± 0.01[c]
Liver	6.88 ± 0.28	3.52 ± 0.11[c]
Brain	6.99 ± 0.14	1.68 ± 0.59[c]

[a] Mice were treated with CsA (75 mg/kg per day) beginning on the day before inoculation. Control mice received the equivalent amount of Cr-E on the same schedule. All mice were inoculated i.v. with *C. neoformans* 145A (3.0 CFU).

[b] Death for mice receiving Cr-E was between 21 and 34 days. Death for mice receiving CsA was at the completion of the experiment at 45 days.

[c] $P < 0.001$; n = 5 per group.

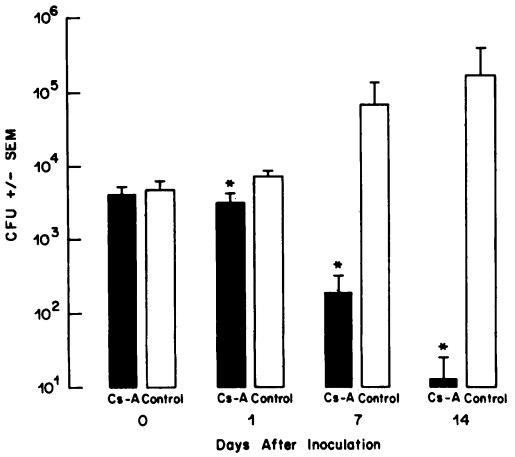

FIG. 3. Effect of CsA on pulmonary clearance of *C. neoformans* 145A inoculated i.t. (10^4 CFU). Mice (n = 5 per group) were given CsA (50 mg/kg per day s.c.; ■) or a control solution containing Cr-E (□) beginning on the day before inoculation. Data are expressed as the total CFU per lung. Symbol: *, $P < 0.001$.

TABLE 2. Effect of CsA on *C. neoformans* in the lungs of mice after i.t. inoculation[a]

Cryptococcal strain	CFU (log_{10}) organ in lungs at:		Animal treatment
	Deposition	Day 4	
145A	3.79 ± 0.12	2.60 ± 0.12[b]	CsA
		4.56 ± 0.05	Control
36556	4.21 ± 0.05	2.62 ± 0.19[b]	CsA
		4.76 ± 0.11	Control
H99	5.41 ± 0.05	4.92 ± 0.02[b]	CsA
		5.83 ± 0.06	Control

[a] Mice received CsA (20 mg/kg per day s.c.) or Cr-E (control solution) equivalent to 20 mg/kg per day beginning on the day before inoculation. $n = 5$ in each group.
[b] $P < 0.001$.

Effect of CsA on survival in mice receiving *C. neoformans* i.t. We examined the effect of CsA on survival following i.t. inoculation of *C. neoformans*. Mice received CsA (50 mg/kg per day) or a control solution beginning on the day prior to i.t. inoculation with *C. neoformans* 145A (10^4 CFU). The first animal in the control group died 40 days after inoculation, and the mean survival time was 51.5 ± 1.1 days (Fig. 4). All animals in the control group were dead by day 55. This survival was similar to that of untreated animals inoculated i.t. with 10^4 CFU in preliminary experiments (data not shown). CFU at death in control mice revealed dissemination from the lung with prominent central nervous system infection (the CFU [log_{10}] was as follows in: lung, 7.13 ± 0.14; spleen, 4.43 ± 0.15; kidney, 4.28 ± 0.38; liver, 4.89 ± 0.27; and brain, 6.96 ± 0.05). All CsA-treated animals survived until the experiment was terminated on day 69, 14 days after the last death in the control group. The CFU in a representative mouse receiving CsA, in CFU (log_{10}), was as follows in: lung, 2.6; spleen, 0.9; kidney, 2.3; liver, 2.0; brain, 1.6.

Effect of CsA on mitogen-induced lymphoproliferation. Lastly, we determined the effect of chronic CsA treatment on the response of splenocytes to the T-cell mitogen concanavalin A. There was a significant reduction in the response to concanavalin A of splenocytes from CsA-treated mice as compared with those from controls (Fig. 5).

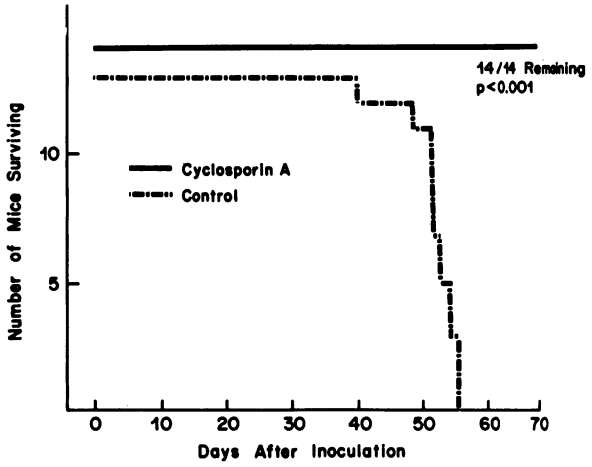

FIG. 4. Effect of CsA on survival after i.t. inoculation of *C. neoformans* 145A (deposition, $4.5 \times 10^3 \pm 0.6 \times 10^3$ CFU). Mice were treated with CsA ($n = 14$; 50 mg/kg per day) or a control solution containing Cr-E ($n = 13$; equivalent to 50 mg/kg per day) beginning on the day before inoculation of *C. neoformans*.

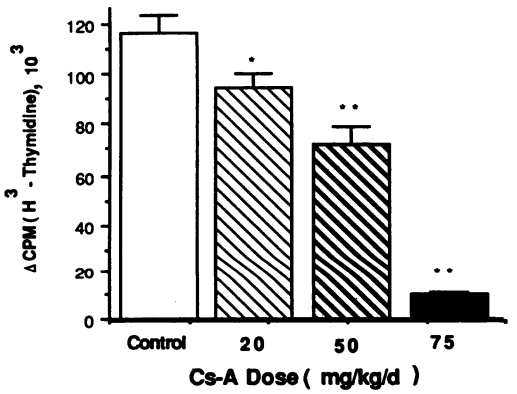

FIG. 5. Effect of CsA on concanavalin A-induced splenocyte proliferation. Mice were treated with CsA (20, 50, and 75 mg/kg per day) for 7 days. The response of splenocytes (3×10^5/well) to 2 μg of concanavalin A per ml was compared with that of splenocytes from controls. The responses of splenocytes from all treated animals were significantly different from those of control splenocytes. Symbols: *, $P < 0.05$; **, $P < 0.001$.

DISCUSSION

Results of this study indicate that CsA enhances survival following both i.v. and i.t. inoculation of *C. neoformans*. Furthermore, CsA enhances the clearance of *C. neoformans* from the lung following i.t. inoculation. The mechanism of enhanced survival and clearance relates at least in part to the direct inhibition of cryptococcal growth as shown by in vitro testing.

C. neoformans 145A was administered both i.v. and i.t. during this study. Intravenous inoculation of organisms has been widely used to study the pathogenesis of cryptococcal infections and may represent a model of fungemia. Following i.v. inoculation, organisms were found in lung, liver, kidney, spleen, and brain. A prominent central nervous system infection was present at death. However, cryptococcosis is likely acquired by the pulmonary route (11, 16). Therefore, i.t. inoculations were used predominantly in these studies. The inoculum size was chosen because in control animals it was associated with widespread dissemination and 100% mortality. Lower inoculum sizes did not uniformly result in dissemination and death. Control animals inoculated i.t. also developed widespread dissemination, although the course of the infection was more prolonged than in the i.v. model. A prominent central nervous system infection was present at death in animals inoculated i.t., as well as those inoculated i.v.

These data demonstrate an anticryptococcal effect of CsA. However, CsA administration has been previously shown to be immunosuppressive. The doses of CsA used in these experiments have been shown to cause T-cell dysfunction in previous studies. Prolonged skin allograft survival and depressed delayed hypersensitivity was demonstrated following administration of 70 and 300 mg of CsA per kg per day, respectively (3, 4). Mice pretreated with 50 mg of CsA per kg per day failed to mount an appropriate skin test response to antigen after they were given T cells capable of mediating delayed-type hypersensitivity (24). A dose of 20 mg of CsA per kg per day has been shown to suppress the response to vaccination with *Listeria monocytogenes* (28). We demon-

strated that all CsA treatment protocols diminished the response of spleen cells to the mitogen concanavalin A following 7 days of CsA treatment. This strongly suggests that the levels of CsA in our experimental animals would have been sufficient to prevent the development of specific immune responses. These data are similar to those given in a previous report (21), in which it was shown that CsA diminished the response of rabbit peripheral blood lymphocytes to concanavalin A. Thus, since growth of *C. neoformans* is likely enhanced by the immunosuppressive effect of CsA, the apparent antifungal effect of CsA is actually less than would be observed if immunosuppression were not also operative in the experimental animals.

Nevertheless, levels of CsA in serum are variable in mice, and it has been proposed (1) that levels in serum be measured in experimental systems when CsA is used. The preparation of CsA we used (Sandimmune IV) has not been used in s.c. administration in mice. Injections of 50 to 75 mg of CsA per kg per day by the s.c. route yielded levels within the range that produced immunosuppression in in vitro experiments with murine lymphoid cells (8, 15). Recently, it has been shown that subcutaneous administration of CsA with the equivalent of 25 mg/kg (in an average 25-g mouse) correlated with a CsA level in serum at 24 h of 0.33 μg/ml (23). This level of CsA was shown to decrease the mortality and neurologic signs in a murine model of experimental allergic encephalomyelitis, and to decrease the production of lymphokine-derived chemotactic factor (23). Despite the likelihood that profound immunosuppression was induced in our mice by CsA, treated animals cleared *C. neoformans* 145A effectively and survived. In contrast, in control animals *C. neoformans* grew at a rate similar to that of untreated mice in previous experiments, and all mice rapidly succumbed to cryptococcal infection.

The level of CsA in blood in human immunosuppressive therapy ranges between 0.1 and 0.3 μg/ml and is 10-fold less than that achieved in murine immunosuppressive therapy when mice receive 50 to 75 mg of CsA per kg per day. In the i.t. inoculation experiment in which 20 mg of CsA per kg per day was used, levels in blood were 0.3 μg/ml, which is in the range of that in transplant patients (7) and the same as that which produces immunosuppression in a murine model of experimental allergic encephalitis. In mice that received 20 mg of CsA per kg per day, we demonstrated effective clearance of three strains of *C. neoformans* from the lungs as compared with that in mice that received a control solution. Levels of CsA in blood were used to select the concentration of CsA in an in vitro assay. Results of the in vitro experiments demonstrated that concentrations of CsA of 0.1 μg/ml were also effective in inhibiting the growth of these same three strains.

The susceptibility of other fungal organisms to CsA has been demonstrated in vitro for *Aspergillus candidus*, *Saccharomyces cerevisiae*, and *Candida albicans* (20). Susceptibility to CsA has also been documented in vitro and in vivo with *Leishmania major* and *Leishmania tropica* (2, 25). In vivo susceptibility to CsA has been shown with *Schistosoma mansoni* (5), *Coccidioides immitis* (13), *Plasmodium* sp. (26), *Dipetalonema vitae* (6), and *Toxoplasma gondii* (17). Of particular relevance to our studies, CsA has not been shown to affect the growth of *C. neoformans* either in vitro or in vivo in two other studies (20, 21).

The failure of the in vitro sensitivity of *C. neoformans* to CsA to be demonstrated in other studies might reflect the assay system. The in vitro susceptibility testing of CsA in our studies was performed by using a commercially available preparation of CsA in Cr-E, which allowed for the more uniform dispersion of CsA. Broth rather than agar was used, and the medium was constantly agitated on an orbital shaker to promote aeration and uniform distribution. By using this assay system, the susceptibility demonstrated in vitro correlated with the enhanced clearance and survival shown in vivo.

Our results differed from those reported previously (21) in a model of *C. neoformans* meningitis in rabbits immunosuppressed with CsA. *C. neoformans* was administered directly into the intrathecal space, while CsA (30 mg per rabbit) was administered i.v. Cryptococcus was cleared less effectively in immunosuppressed rabbits. CsA was not detected in cerebrospinal fluid with an assay which could detect 0.005 μg of CsA per ml. This low level of CsA in cerebrospinal fluid may explain the lack of apparent susceptibility of the organism to CsA in this model. Alternatively, the differences in route of infection and in doses of CsA used may account for the differences in results between these two studies.

In summary, CsA administration prolonged the survival of mice inoculated with *C. neoformans* either i.v. or i.t. The enhanced survival was associated with a reduced burden of *C. neoformans* in lung, spleen, liver, kidney, and brain. Furthermore, following i.t. inoculation of the organism, CsA administration markedly enhanced the clearance of *C. neoformans* from the lungs. Finally, the enhanced survival and clearance correlated with inhibition of growth of *C. neoformans* by CsA in vitro. The susceptibility of *C. neoformans* was documented at levels in serum that are in the therapeutic range for the human transplant population. Further work must be done to define the sensitivity of *C. neoformans* in mice treated after an established infection, particularly when the central nervous system is infected. While these results would have to be extended to studies in humans, these data suggest that patients who now receive CsA may be fortuitously protected against certain infections with *C. neoformans*. Furthermore, it is possible that CsA might be modified to be less immunosuppressive and thus useful in the treatment of patients with cryptococcal infections.

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