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Clearance of *Cryptococcus neoformans* from Immunologically Suppressed Mice

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To assess the effects of cryptococcal antigen-induced immunosuppression on a *Cryptococcus neoformans* infection, CBA/J mice were injected intravenously with saline or suppressive doses of cryptococcal antigen (CneF) at weekly intervals and were then infected with viable *C. neoformans* cells. By the second week after infection, the cryptococcal antigen-injected mice had suppressed anticryptococcal delayed-type hypersensitivity (DTH) responses compared with the responses of the saline-treated, infected control mice. In addition, the immunosuppressed mice had higher numbers of cryptococcal CFU cultured from their lungs, livers, spleens, lymph nodes, and brains than did the control animals. A direct correlation of suppression of the anticryptococcal DTH response and reduced clearance of cryptococci from tissues was also observed after mice were given a single intravenous injection of CneF and infected. To determine whether or not the cryptococcal antigen was specifically reducing the clearance of *C. neoformans* or had a more generalized effect, mice were injected with saline or suppressive doses of CneF, infected with *Listeria monocytogenes*, and then followed daily for 7 days for the clearance of *L. monocytogenes* from spleens and on day 7 for DTH reactivity to *Listeria* antigen. There were no differences between the saline- and CneF-treated mice with respect to anti-*Listeria* DTH responses or clearance of *L. monocytogenes* from spleens, indicating that CneF was not altering natural resistance mechanisms responsible for early clearance of *L. monocytogenes*, nor was the CneF influencing the induction of the acquired immune response which was responsible for the late clearance of the bacteria. Together, these data indicate that the specific suppression of this cell-mediated immune response induced by cryptococcal antigen reduces the ability of the animals to eliminate the homologous organism (*C. neoformans*) but not a heterologous infectious agent, such as *L. monocytogenes*.

Cryptococcosis is a disease caused by an encapsulated yeastlike organism, *Cryptococcus neoformans*. This organism tends to shed antigens into body fluids, and patients with disseminated cryptococcosis generally have high levels of cryptococcal antigen detectable in their spinal fluid and/or serum (9, 13, 33). High or increasing cryptococcal antigen titers are indicative of progressive disease, whereas decreasing titers are accompanied by clinical improvement (9, 13). Another feature frequently displayed by patients with disseminated cryptococcosis is a depressed cell-mediated immune (CMI) response to cryptococci or cryptococcal antigens (8, 14, 34). It is well established that the CMI response is a crucial component of the host defense against *C. neoformans* (3, 15, 21). In humans, the importance of CMI function in protection against this fungus is emphasized by the high incidence of disseminated cryptococcosis in patients with acquired immunodeficiency syndrome, with malignancy, or on steroid or immunosuppressive chemotherapy (7, 11, 19). Depressed CMI responses are not only observed in immunocompromised individuals but have been reported in disseminated cryptococcosis patients who appear to be otherwise immunologically normal (8, 14, 34). The association of high levels of cryptococcal antigen and depressed CMI reactivity to cryptococcal antigen in patients with progressive disease suggests that the antigen may play a role in modulating the immune response.

With the mouse model, it has been well documented that cryptococcal antigen given intravenously (i.v.) or intraperitoneally to immunocompetent animals, in fact, does induce suppression of anticryptococcal CMI responses (1, 2, 12, 18, 27–32). This suppression, as determined by reduced delayed-type hypersensitivity (DTH) responses, has been shown to be specific for cryptococcal antigen and to be mediated by a circuit of suppressor cells and soluble suppressor factors (12, 18, 27, 28, 30–32). In addition, treatment of normal mice with soluble cryptococcal capsular polysaccharide antigen has been reported to induce T cells which, upon restimulation with cryptococcal antigen but not heterologous antigens, produce a soluble factor capable of reducing the ability of macrophages to phagocytize cryptococci as well as *Saccharomyces cerevisiae* (1, 2).

Although cryptococcal antigen-induced suppressor cells have been shown to specifically suppress the anticryptococcal DTH responses in that they do not suppress DTH responses to dinitrofluorobenzene or purified protein derivative of *Mycobacterium tuberculosis* (30, 31), the effects of cryptococcal antigen on the development of the protective immune response during an infection with cryptococci or a heterologous infectious agent have not been determined. Therefore, the primary purpose of the present study was to assess the influence of cryptococcal antigen treatment on DTH responses and organism clearance from tissues of mice infected with either *C. neoformans* or the heterologous organism *Listeria monocytogenes*.

MATERIALS AND METHODS

Mice. Inbred CBA/J female mice, purchased from Jackson Laboratory, Bar Harbor, Maine, were used at 7 to 10 weeks of age in these studies.

Cryptococcal antigen. A cryptococcal culture filtrate (CneF) antigen was used for the induction of suppression and footpad challenge. The antigen was prepared by the

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method of Cauley and Murphy (3), and the lot of antigen used in this study had a protein concentration of 11.3 mg/ml, as determined by the procedure of Lowry et al. (23) and as modified by Miller (25), and a carbohydrate concentration of 3.3 mg/ml, as assayed by the phenol-sulfuric acid method (10).

Infection studies with *C. neoformans*. CBA/J mice were injected i.v. at weekly intervals for 10 weeks with 0.4 ml of either sterile physiological saline (control group) or CneF antigen (test group). One week after the first saline or CneF injection, the mice were infected intranasally (i.n.) with 5×10^4 viable *C. neoformans* cells of isolate 184 (22). At weekly intervals after infection, three mice were randomly selected from each treatment group and were footpad challenged with 30 μl of CneF to determine their DTH responses (3). Footpad swelling was measured 24 h after challenge, and then the mice were necropsied to assess the numbers of cryptococcal CFU in lungs, livers, spleens, lymph nodes, and brains as previously described (3, 22). This experiment was repeated three times.

In a second study, mice were injected i.v. with a single dose of saline or CneF (0.4 ml) 1 week before the animals were infected i.n. with 4×10^4 viable cryptococci. At weekly intervals for 4 weeks after infection, three mice from each group were randomly selected, footpad challenged with CneF, and necropsied 24 h after footpad challenge to determine the numbers of cryptococcal CFU in the same tissues listed above. This experiment was repeated twice.

Since not every mouse within a group had all culturally positive tissues, it was of interest to compare the saline- and CneF-treated groups with respect to the mean percentages of positive cultures for each tissue and from all of the tissues combined over the 9-week period. The mean percent culturally positive tissues was determined by taking the mean of the percentages of mice from which *C. neoformans* was isolated for the tissue in question for all time periods throughout the study. For calculation of the mean percentage of total culturally positive mice, a similar calculation was made as that which was made for individual tissues, and if an animal had one or more culturally positive tissues, it was considered to be positive.

Infection studies with *L. monocytogenes*. To determine the effects of i.v. administered CneF on a heterologous infection, mice were injected with CneF i.v. by using several different injection protocols and were then infected with *L. monocytogenes*, which causes an acute infection in contrast to the chronic disease caused by *C. neoformans*. Similar results were obtained with all of the different protocols, so only the two representative procedures which were used to obtain the data presented in this report are described. In one case, 7- to 8-week old CBA/J mice were injected i.v. with 0.4 ml of saline or CneF on days −21, −14, and −7 before the animals were infected i.v. with 10^4 viable *L. monocytogenes* cells on day 0. In the other case, mice were given 0.4 ml of CneF i.v. on days −14, −7, and 0 before they were infected with 10^4 *L. monocytogenes* on day 0. Four animals from each treatment group were randomly selected on a daily basis after infection, and the numbers of *L. monocytogenes* CFU per spleen were determined by plating the diluted homogenates of the spleens on nutrient agar. On day 7 after infection, five mice from each treatment group were footpad tested with 30 μl of *Listeria* intracellular product (17), which was kindly provided by Robert Kearns, University of Dayton, Dayton, Ohio. The increase in footpad swelling was measured 24 h after footpad challenge. For a negative footpad test control, uninfected mice were footpad challenged with *Listeria* intracellular product. These experiments were repeated twice.

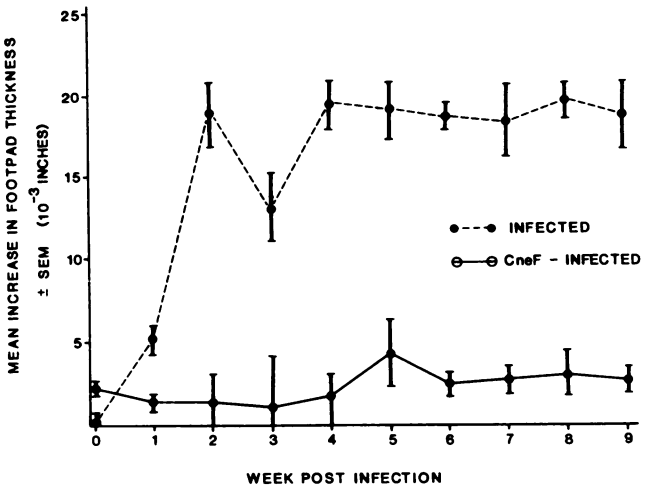

FIG. 1. Temporal profile of DTH responses elicited by CneF in mice infected i.n. on day 0 with 5×10^4 cryptococci. Open circles represent the DTH reactions at various times after infecting mice that were injected intravenously with 0.4 ml of CneF at weekly intervals beginning 1 week prior to infection and continuing until the week before the DTH measurements were made. Closed circles denote DTH responses of infected mice treated with 0.4 ml of physiological saline on the same schedule as that used for the CneF treatment. This experiment was repeated three times, and three mice were assessed for DTH reactivity at each time period. Vertical bars indicate the SEMs.

Statistical analyses. The means and standard errors of the means (SEMs) were determined for the various parameters

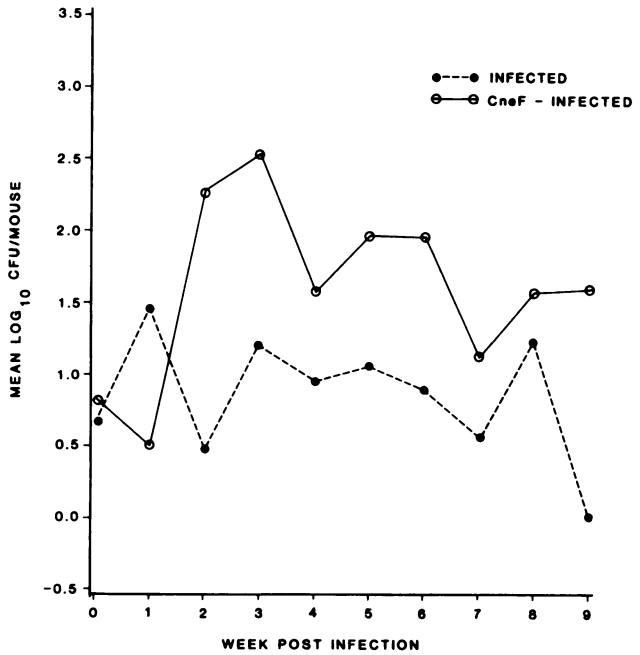

FIG. 2. Cumulative numbers of cryptococcal CFU isolated in three experiments from brain, liver, lungs, lymph nodes, and spleen over the course of a 9-week period after infecting mice i.n. with 5×10^4 viable *C. neoformans* cells. Treatment groups are the same as those described in the Fig. 1 legend.

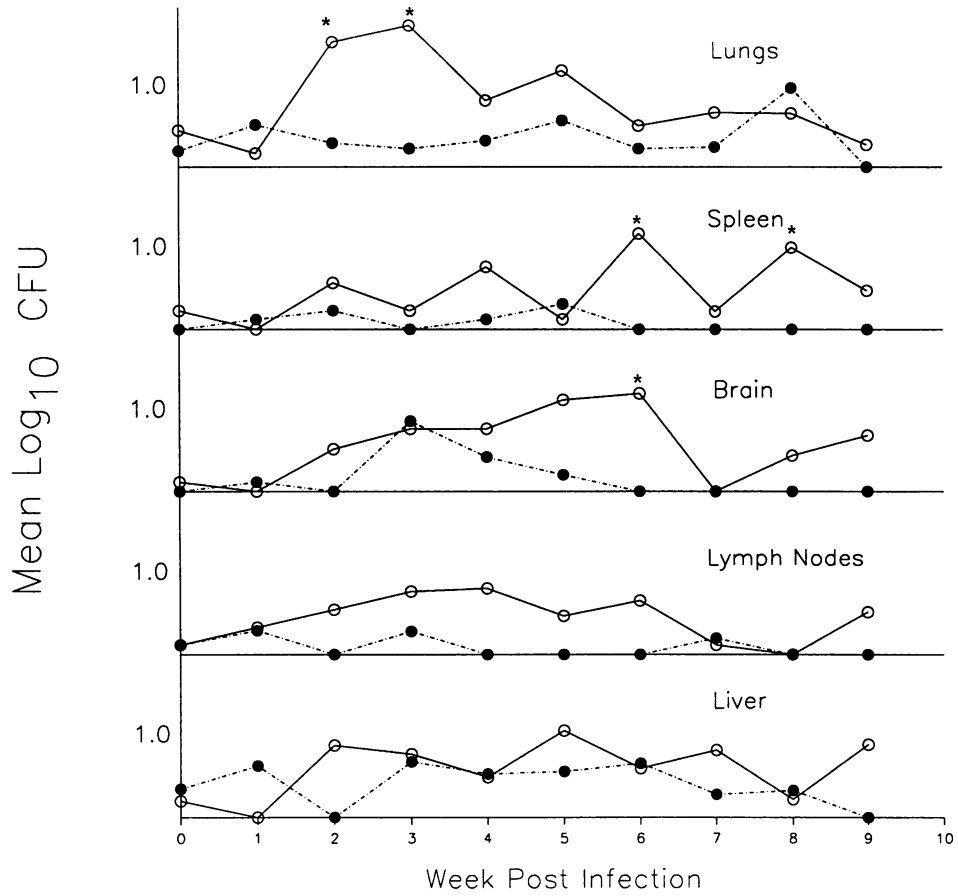

FIG. 3. Temporal display of cryptococcal CFU isolated from the individual tissues of mice treated with saline or CneF and infected i.n. with 5×10^4 viable *C. neoformans* cells. Treatment groups are the same as those described in the Fig. 1 legend. An asterisk indicates that the results for the saline and CneF groups were significantly different at a 95% confidence level.

studied for each group of mice. The unpaired Student's *t* test was used to analyze the data. When comparing two groups, a *P* value of 0.05 or less was considered to indicate that results for the two groups were significantly different.

RESULTS

Anticryptococcal DTH reactivity and organism clearance from mice treated with saline or CneF and infected with *C. neoformans*. Temporal profiles of DTH reactivity in mice were followed in three separate experiments in which the animals were given physiological saline or CneF i.v. at weekly intervals and infected i.n. with *C. neoformans* 1 week after the first injection of antigen. The mice treated with CneF displayed significantly suppressed DTH responses compared with the DTH responses of mice treated with saline from 2 weeks after infection to the end of the 9-week experimental period (Fig. 1). Also, beginning at 2 weeks after infection and continuing throughout the 9-week study, the mean numbers of cryptococcal CFU isolated from lungs, spleens, brains, lymph nodes, and livers of the CneF-treated mice were higher than those from mice treated with saline; however, the mean numbers of CFU from the CneF-treated, infected mice were significantly higher than the mean numbers of CFU from the saline-treated, infected group only at weeks 2, 3, 6, and 9 (Fig. 2).

The mean $\log_{10}$ cryptococcal CFU cultured from each tissue over the experimental period are shown in Fig. 3. Immediately and 1 week after infection, there were no significant differences between the mean CFU isolated from the CneF-treated mice and those of the controls for any tissue assayed. However, at 2 and 3 weeks after infection, the lungs of the CneF-treated mice had significantly higher mean numbers of cryptococcal CFU than did the saline controls for the respective time periods. Then, by 6 weeks after infection, the spleens and brains of the CneF-treated mice began to show significantly higher levels of cryptococci than did the respective tissues from the saline-treated group. The mean numbers of cryptococci cultured from the spleens of the CneF-treated mice were also significantly higher than the controls at 8 weeks postinfection. The lymph nodes of mice treated with CneF had higher mean numbers of cryptococcal CFU ($\log_{10}$ CFU range, from 0.47 to 0.81) than did the control animals ($\log_{10}$ CFU did not exceed 0.28) from 2 through 6 weeks after infection; however, the results between the two groups were not significantly different at the 95% confidence level. The livers of the saline- and CneF-treated mice did not contain significantly different mean numbers of CFU at any time period, although at 2, 5, 7, and 9 weeks the mean values for the CneF group were higher than were those for the control group. There was never a time after 2 weeks into the infection that the saline-treated mice had higher mean numbers of CFU in the livers than did the CneF-treated group.

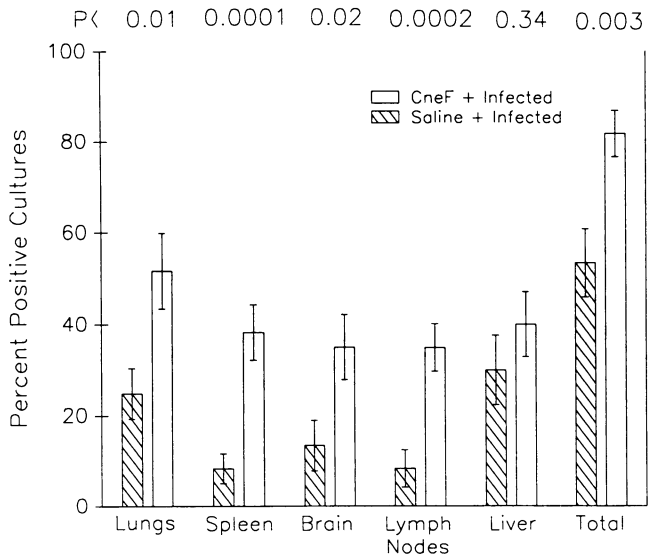

FIG. 4. Comparisons of the percentages of culturally positive tissues obtained over the 9-week period from mice treated with suppressive doses of CneF or saline and infected with *C. neoformans*. Slashed bars are saline-treated and infected mice; open bars are CneF-treated and infected mice. Bars represent the SEMs.

Because at each time period there were some tissue samples that were not culturally positive for *C. neoformans*, it seemed important to determine the mean percentage of positive samples that were obtained from each tissue sample from saline- or CneF-treated mice over the entire course of infection. When the saline- and CneF-treated groups were compared in this manner by using the data obtained from all three experiments, the animals which were treated with CneF and infected had significantly higher percentages of culturally positive lungs, spleens, brains, and lymph nodes than did the control groups which were treated with saline and infected (Fig. 4). The only organ in which the percentages of positive cultures were not significantly different between the saline- and CneF-treated groups was the liver; however, in all three experiments, the mean percentages of positive cultures were higher for the CneF-treated group compared with the saline-treated group (Fig. 4). The mean percentage of culturally positive mice in the CneF-treated group was significantly higher than the mean percentage of culturally positive mice in the saline-treated group (Fig. 4).

Another experiment similar to that described above was performed, but instead of giving saline or CneF weekly, only one i.v. injection of saline or CneF was given 7 days before the mice were infected i.n. with 4×10^4 viable *C. neoformans* cells. In this experiment, the DTH responses of the mice treated with CneF were also significantly suppressed compared with the responses in the saline-treated control group ($P < 0.005$) by 5 weeks after CneF treatment or 4 weeks after infection (Fig. 5A), and the mean numbers of cryptococcal CFU in the immunologically suppressed group were significantly higher than the numbers of CFU cultured from the control group ($P < 0.05$) (Fig. 5B). When the mean percentages of culturally positive mice were calculated and compared for the two groups over the 4-week period, the animals that were treated with CneF and infected had approximately three times the percentage of positive cultures as that of the saline-treated, infected group, and the difference was significant ($P < 0.05$).

Anti-*Listeria* DTH reactivity and clearance of *L. monocytogenes* from tissues of mice treated with CneF or saline. In previous studies (30, 31), it has been demonstrated that the suppression of the DTH response to CneF is specific for cryptococcal antigen, in that suppressor cells induced by CneF would not suppress DTH responses to dinitrofluorobenzene or purified protein derivative of *M. tuberculosis*. In this study, I was interested in determining whether CneF antigen was specifically affecting the clearance of cryptococci from the murine tissues or whether the cryptococcal antigen had a more generalized effect and altered the clearance of organisms other than *C. neoformans*. To assess this, mice were given three i.v. injections of CneF at weekly intervals before the animals were infected with *L. monocytogenes*. The protocol of three CneF injections was selected because it was after three injections of CneF in the studies on *C. neoformans* clearance that notable differences in the anticryptococcal DTH responses and clearance of cryptococci from tissues could be detected between the CneF-treated and saline-treated mice. Since it was desirable for the mice in the *Listeria* clearance studies to be subjected to similar amounts of CneF before being infected with *L. monocytogenes*, three weekly injections were used to assess the effects of CneF on clearance of the heterologous organism. Because I was not certain of the differences in the effects of ending the CneF injections on the day of infection versus ending the CneF injections 1 week before infection, two different protocols were used. In one case, mice were injected with CneF or saline i.v. at three weekly intervals beginning 21 days and ending 1 week before introduction of the infecting dose of *L. monocytogenes*; and in the other case, the animals were given three weekly injections of CneF or saline beginning 14 days prior to infection, so that the last dose of CneF was given on the same day as the infective dose of the heterologous organism. The mean anti-*Listeria* DTH responses of the animals that received CneF were comparable to the mean DTH responses of saline-treated,

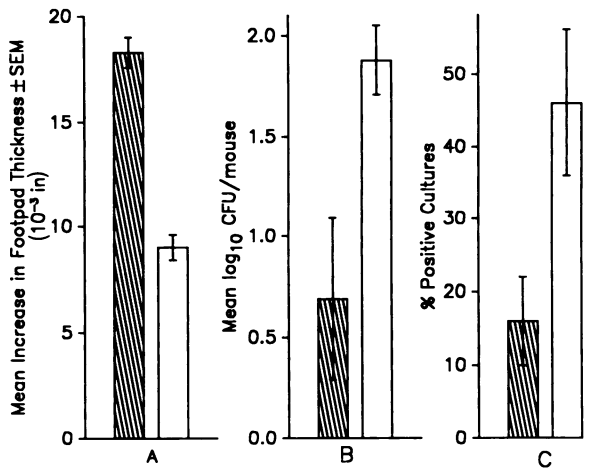

FIG. 5. Effects of a single i.v. injection of saline or CneF on DTH responses and clearance of *C. neoformans* following an i.n. infection with *C. neoformans*. Mice were given 0.4 ml of saline or CneF 1 week before an i.n. infection with 4×10^4 *C. neoformans* cells. DTH responses (mean increase in footpad thickness) (A); mean $\log_{10}$ CFU (B); and mean percent positive *C. neoformans* cultures isolated from lungs, livers, spleens, brains, and lymph nodes (C) were determined 4 weeks after mice were infected. The data were combined from two experiments in which three mice per group per experiment were used. Slashed bars are saline-treated and infected mice; open bars are CneF-treated and infected mice. Bars represent the SEMs.

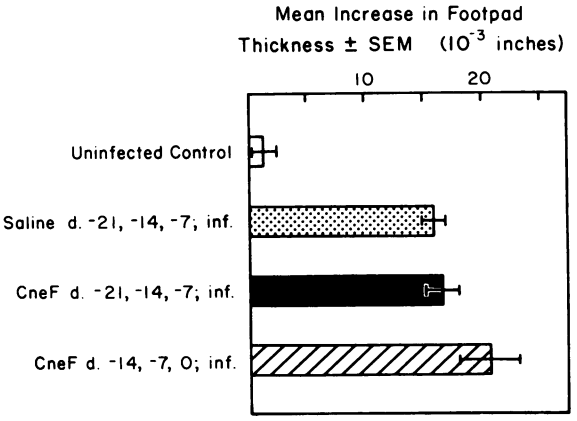

FIG. 6. DTH responses elicited by *Listeria* intracellular product in mice pretreated with three weekly injections of either CneF (solid and hatched bars) or saline (dotted bars) and then infected (inf.) with *L. monocytogenes* on day 0 and footpad challenged on day 7. The data were pooled from two experiments in which five mice were used per group per experiment. Bars indicate the SEMs. d. indicates days.

infected mice (Fig. 6). Since the DTH data from both saline-treated, infected groups were similar, data from only one of the two saline control groups are presented in Fig. 6. Three injections of CneF antigen did not alter the clearance of *L. monocytogenes* from the spleens of the infected mice, as indicated by the similarities in the mean numbers of *L. monocytogenes* CFU isolated from the control and CneF-treated mice at each time period (Fig. 7).

DISCUSSION

For years, investigators have noted a correlation between the level of cryptococcal antigen in body fluids and the severity of disease in patients with cryptococcosis (9, 13, 33). High or increasing cryptococcal antigen titers in serum or spinal fluid are indicative of progressive disease, and conversely, decreasing titers signal clinical improvement (9, 13). Furthermore, it has been reported that patients who have disseminated cryptococcosis or who have recovered

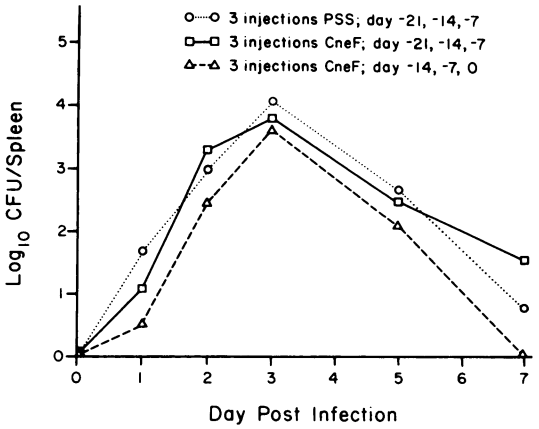

FIG. 7. Numbers of *L. monocytogenes* CFU from spleens of mice treated with three weekly injections of CneF (□ and △) or saline (○) prior to challenge with viable *L. monocytogenes*. Data from two experiments in which there were four mice per group per experiment are represented.

from cryptococcosis frequently display depressed CMI responses to cryptococcal antigen compared with normal individuals who have been exposed to cryptococci (8, 14, 34). These observations suggest that cryptococcal antigen may be adversely affecting the anticryptococcal CMI response, which is the primary protective immune response (3, 15, 21); therefore, my laboratory has been interested in how cryptococcal antigen influences the anticryptococcal CMI response. To gain insights into this, a mouse model has been used to define the effects of cryptococcal antigen on the primary protective immune response against *C. neoformans* (12, 18, 27–32). In this animal model, antigen-specific suppression of the anticryptococcal CMI response, as measured by the DTH reactivity, can be readily induced by administering i.v. cryptococcal antigen which has been prepared either in vivo (serum from *C. neoformans*-infected mice with high cryptococcal antigen titers) (29, 30) or in vitro (CneF antigen) (28, 30, 32). Other investigators have also documented that cryptococcal antigen can induce suppressor T cells, which, upon restimulation with cryptococcal antigen but not heterologous antigens, diminish the ability of macrophages to phagocytize not only cryptococci but also other organisms (1, 2). These studies imply, but do not directly demonstrate, that the host with high levels of cryptococcal antigen may be compromised in its ability to eliminate *C. neoformans*. In the present study, using a more direct approach, it has been shown that cryptococcal antigen given i.v. at levels that induced suppression of the anticryptococcal DTH response (Fig. 1 and 5A) also reduced the ability of mice to clear *C. neoformans* from infected tissues (Fig. 2, 3, 4, and 5B and C).

The first experiments that demonstrated CneF adversely affected the elimination of *C. neoformans* from tissues were designed to assess the numbers of cryptococcal CFU in lungs, spleens, brains, lymph nodes, and livers of mice that were given cryptococcal antigen at weekly intervals to maintain a reasonably high level of cryptococcal antigen in body fluids. In addition, the mice were infected by the i.n. route, which has been shown to result in a chronic infection accompanied by positive DTH reactivity to CneF (22). In many respects, murine cryptococcosis induced in this manner is similar to human cryptococcosis (22). For the present study, the first injection of cryptococcal antigen was given 1 week prior to infection to allow time for the induction of first-order T suppressor cells before the mice were subjected to viable cryptococci (30, 32). As expected, mice treated with CneF did not develop anticryptococcal DTH reactivity after an i.n. instillation of cryptococci, whereas the saline-treated controls developed strong DTH responses to CneF by 2 weeks following introduction of the infectious dose of *C. neoformans* (Fig. 1). The DTH data obtained for the saline-treated, infected group of mice are consistent with previous observations of DTH reactivity in mice infected i.n. with 10^3 *C. neoformans* cells but not treated with saline (22).

The general patterns of mean CFU counts over the 9-week period for total organs or individual organs such as lungs, spleens, brains, and livers from the saline-treated, *C. neoformans*-infected group were similar to those reported for the respective tissues by Lim et al. (22). In mice that were treated with CneF and infected, the increases in cryptococcal CFU over the control levels appeared in waves in the various tissues. At 2 and 3 weeks after infection, when the mean DTH responses in control mice were relatively high and DTH responses in CneF-treated mice were greatly suppressed (Fig. 1), the lungs of the CneF-treated animals showed significantly increased mean numbers of cryptococ-

cal CFU over the control levels (Fig. 3). The next tissues to show significant increases over the controls in mean organism counts were the spleens and brains. These data suggest that there was a greater degree of proliferation of the organisms in the lungs early after infection (2 to 3 weeks), and then dissemination occurred to a larger extent over an extended period of time in the animals that had suppressed DTH responses compared with the animals that mounted a strong DTH responsiveness. Although there were not significant differences in the mean numbers of CFU isolated from the lymph nodes of CneF-treated mice compared with those from the lymph nodes of saline-treated mice, there were significant differences between the two groups of mice with respect to the mean percentages of culturally positive lymph nodes. It should be pointed out that the CneF-treated mice had higher numbers of CFU, although not significantly higher at the 95% confidence level, in their lymph nodes for 5 consecutive weeks than did the saline control animals. It is possible that significant differences were not achieved at this level of confidence because the numbers of CFU isolated from the lymph nodes and the percentage of culturally positive lymph nodes, in general, were lower than the comparable parameters of other tissues; therefore, there was more overlap with the control population. The organism counts in the livers of the two groups of mice were similar throughout the 9-week study. One might expect to find that the numbers of cryptococci would be held in check in lymphoid organs and tissues with a substantial amount of lymphoid tissue, especially in animals that mount a CMI response to the cryptococci, and basically this is what was observed in these studies.

The second set of experiments done in this investigation confirmed that i.v.-administered CneF would affect the clearance of cryptococci. In this case, however, a single injection of CneF was given which suppressed the mean anticryptococcal DTH response by 51% of the control levels; however, even 51% suppression of the CMI response was sufficient to demonstrate a negative effect on the clearance of cryptococci from infected tissues (Fig. 5B and C). The combined data from these two sets of experiments, in which CneF was given i.v. before infection with *C. neoformans*, clearly demonstrate that cryptococcal antigen not only reduces the DTH reactivity but, in addition, it adversely affects elimination of the organism from tissues. Thus, it seems reasonable to predict that cryptococcal antigen in body fluids of cryptococcosis patients can induce the suppressor cell cascade, which then has effects on the immune response and the clearance of the organism similar to those reported here.

In previous studies in which CneF was shown to induce suppression of the CMI response, the suppression was antigen specific (30, 31); therefore, in this investigation I was interested as to whether or not the cryptococcal antigen would affect the clearance of a heterologous organism. *L. monocytogenes* was selected as the heterologous organism to use for determining the specificity of the CneF-induced suppression on clearance mechanisms, because the normal clearance pattern and the mechanisms involved in the clearance of *L. monocytogenes* from murine spleens have been explicitly defined (4–6, 16, 20, 24, 26, 35, 36). For example, after a primary infection with *L. monocytogenes*, macrophages are primarily responsible for the early natural or innate defenses (5, 26), and then after cell-mediated immunity develops at about 3 to 4 days of infection, the activated immune system plays the major role in eliminating the facultative intracellular parasite (6, 16, 20, 24, 35). Furthermore, early ingestion of *L. monocytogenes* by macrophages is most likely required for satisfactory antigen presentation for the induction and development of the anti-*Listeria* CMI response (35, 36). Therefore, it could be reasoned that if suppressive doses of CneF were affecting either innate or immune resistance mechanisms nonspecifically, one should be able to detect the effects by noting changes in the early (days 0 to 3) and/or late (days 3 to 7) clearance patterns of *L. monocytogenes* from spleens. In addition, if the normal function of the macrophages in antigen presentation for induction of the anti-*Listeria* CMI response was affected by CneF, one should be able to detect this by seeing an alteration in the development of the anti-*Listeria* DTH response and late clearance of *L. monocytogenes* in mice that were treated with CneF and then infected with *L. monocytogenes*. Since differences were not observed in anti-*Listeria* DTH responses or clearance patterns of *L. monocytogenes* from spleens of mice that were treated with CneF and infected compared with DTH responses and clearance patterns of mice treated with saline and infected with *L. monocytogenes*, I concluded that CneF was specifically regulating the anticryptococcal CMI response and that this specific down-regulation of anticryptococcal cell-mediated immunity was detrimental to the host by obstructing immune mechanisms of clearance. These data, in combination with data from earlier studies showing that an i.v. injection of CneF suppresses the DTH response to CneF but not to two heterologous antigens, dinitrofluorobenzene or *M. tuberculosis* (30, 31), clearly demonstrate that cryptococcal antigen induces suppression that is specific for *C. neoformans* whether one measures the suppression in terms of depressed DTH responses or the clearance of organisms from tissues.

The finding that cryptococcal antigen is specifically regulating the protective immune response against *C. neoformans* suggests that future studies should be focused on developing means of interfering with the down-regulation of the anticryptococcal CMI response. It appears from this study that if suppression of the anticryptococcal CMI response could be prevented or reversed, then the host could more effectively deal with a cryptococcal infection.

ACKNOWLEDGMENTS

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