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Immunology

Antibody-mediated protection in mice with lethal intracerebral *Cryptococcus neoformans* infection

(monoclonal antibody/blood–brain barrier/fungus)

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ABSTRACT **The fungus *Cryptococcus neoformans* is an important opportunistic pathogen for patients with AIDS. *C. neoformans* infections frequently involve the brain and are often fatal. In the setting of AIDS *C. neoformans* infections are incurable and new treatment strategies are urgently needed. Passive administration of antibody is a potential therapeutic option for the prevention and treatment of *C. neoformans*. The IgG1 murine monoclonal antibody 2H1 to the capsular polysaccharide of *C. neoformans* was studied for its ability to modify the course of lethal intracerebral cryptococcal infection in mice. Intraperitoneal administration of antibody 2H1 resulted in small, yet significant, prolongations in the average survival of mice given intracerebral infection and reduced the number of *C. neoformans* colonies in brain tissue. Histopathological examination of brain tissues revealed a diffuse cryptococcal meningitis with fewer organisms in the brains of mice that received antibody 2H1 than in the control group. Thus, systemic administration of a monoclonal antibody can modify the course of lethal intracerebral *C. neoformans* infection in mice by prolonging survival and decreasing fungal burden in brain tissues.**

Cryptococcus neoformans is a yeast-like fungus which causes life-threatening infections in 5–13% of patients with AIDS (1–3). The majority of human infections involve the central nervous system as meningoencephalitis (4–6). In patients with AIDS, *C. neoformans* infections are usually incurable because antifungal therapy cannot eradicate the fungus in the setting of profound immunosuppression (7). As a result, AIDS patients who survive the initial infection require lifelong suppressive therapy with antifungal drugs to reduce the risk of relapse (8).

Cellular immunity is believed to provide the primary defense mechanism against *C. neoformans* (9–11). However, the complement system is also an important host defense mechanism (12–16), and various observations suggest a role for antibody in protection. Capsule-specific antibody efficiently promotes phagocytosis by macrophages (17–19), fungal killing by mononuclear cells (20–22), and fungistasis by natural killer cells (23, 24) *in vitro*. Patients with cryptococcal infections are more likely to survive if they have serum antibodies to *C. neoformans* (25); recovery from cryptococcal infection has been associated with intrathecal production of antibody (26), intrathecal antibody production is temporally associated with a decrease in brain fungal counts in a rabbit model (27), and passive administration of capsule-specific polyclonal (28) or monoclonal (29–31) antibodies can prolong the survival of lethally infected mice and reduce the tissue fungal burden (32).

C. neoformans is unusual among pathogenic fungi in that it has a large polysaccharide capsule. Like the polysaccharide capsules of encapsulated bacteria it is antiphagocytic (18), poorly immunogenic (33–36), and causes immune paralysis (37–39). Like encapsulated bacteria, the capsule in *C. neoformans* is important for virulence (40, 41). Both *C. neoformans* and encapsulated bacteria (42, 43) are frequent pathogens in AIDS patients. The susceptibility of AIDS patients to encapsulated bacteria may result from defects in antibody immunity (44, 45). AIDS patients are also deficient in IgG antibody to *C. neoformans* capsular polysaccharide (46). Since capsule specific IgG is a major opsonin for the fungus in human sera (17), a defect in antibody immunity may contribute to the susceptibility of AIDS patients to *C. neoformans*.

In the pre-antibiotic era, infections with some encapsulated bacteria were successfully treated by the administration of immune sera (47, 48). Intraspinal administration of horse immune serum was effective therapy for meningococcal meningitis (49), establishing a historical precedent for the treatment of a brain infection with antibody alone. Interest in treating *C. neoformans* infections with antibody dates to at least 1925, when Shapiro and Neal (50) used intraspinal administration of rabbit antiserum to treat a boy with cryptococcal meningitis. Unfortunately, the patient did not tolerate repeated injections of heterologous serum and passive antibody therapy was discontinued (50). In 1959 Littman (51) used passive administration of human gamma globulin as an adjunct to antifungal therapy with amphotericin B and reported encouraging results in a small number of patients.

While the role of antibody in the resistance of immunocompetent hosts remains controversial, the AIDS epidemic has renewed interest in using antibody for prevention and treatment of *C. neoformans* infections in immunosuppressed individuals (22, 29, 30, 52, 53). The observation that antibody to *C. neoformans* can enhance the efficacy of amphotericin B (53, 54) suggests a role for antibody in therapy. However, the existence of the blood–brain barrier raises the theoretical concern of whether systemic antibody will be effective in infections of the central nervous system. We have studied this problem in a mouse model of *C. neoformans* intracerebral (i.c.) infection. Systemic administration of capsule-specific IgG1 monoclonal antibody (mAb) modified the course of i.c. *C. neoformans* infection by prolonging survival and decreasing fungal burden in brain tissue.

MATERIALS AND METHODS

mAbs. mAb 2H1 (IgG1) was generated from a BALB/c mouse immunized with *C. neoformans* glucuronoxylomannan conjugated to tetanus toxoid (35). mAb 2H1 binds to the

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Abbreviations: i.p., intraperitoneal(ly); i.c., intracerebral(ly); mAb, monoclonal antibody.

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four cryptococcal serotypes (35); utilizes a heavy-chain variable-region gene from the 7183 family with J_H2, $V_\kappa5.1$, and $J_\kappa1$ genetic elements (31); and has been shown to significantly prolong the survival of mice infected intraperitoneally (i.p.) with *C. neoformans* (30). mAb ascites was made in the hybridoma facility at our institution by i.p. injection of 10^7 hybridoma cells into pristane-primed BALB/c mice. mAb concentration was determined by ELISA relative to standards of the same isotype.

Protection Against i.c. Infection. A/J and BALB/c female mice aged 6–8 weeks were obtained from the National Cancer Institute. A/J mice are more susceptible to *C. neoformans* than BALB/c mice, possibly due to complement deficiency (55). American Type Culture Collection (Rockville, MD) *C. neoformans* serotype D strain 24067 was used for i.c. infection because of our prior experience with it (30). *C. neoformans* was grown in Sabouraud's dextrose broth (Difco) at 37°C. The yeast cells were washed three times in sterile phosphate-buffered saline, pH 7.2 (PBS), resuspended in PBS, and counted in a hemocytometer. Plating efficiencies in Sabouraud's dextrose agar were 40–60%. The relatively low plating efficiencies reflect our practice of counting a budding yeast as two organisms. Since a budding yeast results in only one colony, the agar colony counts are lower than the hemocytometer counts.

The technique used for i.c. infection has been described (56, 57). Mice were anesthetized with methoxythyflourane via inhalation and 50 μl of *C. neoformans* suspension in PBS was injected into the midline of the cranium with a 27-gauge needle. Animals dying within the 24 hr following i.c. inoculation were considered to have died from cerebral trauma. Overall, the mortality from the i.c. injection was <1%. No mice died from i.c. injection in the experiments shown here. Mice were observed at least once and often twice daily. mAb 2H1 was administered i.p. as ascites. Control animals received i.p. injection of the same volume of PBS or, in one experiment (no. 5), ascites containing the IgG1 mAb 36-65, which reacts with *p*-azophenylarsonate but not with cryptococcal polysaccharide. In previous experiments, we have seen no difference between PBS and control myeloma ascites (30), and other investigators have used saline in their control groups as well (15, 32). The fungal burden in brain and lung was determined by homogenizing the organs in PBS, plating the suspension in Sabouraud's agar, and counting colonies. Microscopic inspection of agar plates immediately after the homogenate was plated revealed only single, heavily encapsulated *C. neoformans* on the agar surface. Thus, the reduction of tissue colonies mediated by mAb 2H1 (see below) was not a result of yeast aggregation in tissue homogenates by residual serum antibody.

Statistical Analysis of Survival Data. Survival data were analyzed by Chee Jen Chang of the biostatistics division of our institution, using a log-rank analysis program [SAS Institute (Cary, NC) statistical package].

Histopathologic and Immunohistochemical Studies. Mice were sacrificed by cervical dislocation 4 days after infection. Brain tissue was removed from the cranium and fixed in phosphate-buffered 3.7% formaldehyde for histopathologic examination. Paraffin-embedded tissues cut at 5 μm were stained with hematoxylin and eosin and periodic acid/Schiff reagent for light microscopic examination. Additional paraffin-embedded tissues were stained with mAb 2H1 and with mAbs MOPC 21 (IgG1, unknown specificity) and 2D2 (IgG2a, murine adenovirus-specific) as controls, using the Vector ABC kit (Vector Laboratories) with 3,3′-diaminobenzidine (Vector Laboratories).

RESULTS

The i.c. administration of 1.0–5.0 × 10^3 *C. neoformans* strain ATCC 24067 to mice resulted in a rapidly lethal infection. Within 3–4 days of infection, the mice manifested lethargy, ataxia, and unkempt fur. The i.c. infected mice developed bulging foreheads, presumably reflecting hydrocephalus and swelling of the relatively thin murine skull (5), a finding also described by others (5, 58, 59).

To determine the efficacy of mAb 2H1 in modifying the course of i.c. *C. neoformans* infection, we administered 2H1 i.p. before and after infection. In experiments 1–3, A/J mice were given 1.0 mg of mAb 2H1 i.p. 4–6 hr prior to i.c. infection (Table 1). In experiment 4, BALB/c mice were given 1.0 mg of mAb 2H1 i.p. ≈15 hr after i.c. infection (Table 1). In experiment 5, A/J mice were given 1.0 mg of mAb 2H1 i.p. 24 hr before i.c. infection (Table 2). mAb administered i.p. appears in serum within 6 hr and reaches serum levels comparable to those achieved by intravenous injection after 48 hr (L. Zuckier and M.D.S., unpublished data).

In experiment 1 the average survival was 4.7 days for the control group and 16.5 days for the mAb 2H1-treated group. Survival curves are shown in Fig. 1. The difference in the average survival for the control and mAb 2H1 groups was highly significant, $P = 0.0001$ (Table 1).

Experiment 2 was designed to repeat experiment 1, but in this experiment the administration of mAb 2H1 resulted in a much smaller effect on survival. There was a trend toward increased survival for the mAb 2H1 group that was not statistically significant (Table 1). In addition to the mice studied for survival, two groups of five mice receiving mAb 2H1 or PBS were sacrificed 4 days after infection to determine brain tissue *C. neoformans* colony counts and for histopathologic studies (see below). Despite the small effect of mAb 2H1 on average survival in this experiment, the group that received mAb 2H1 had an ≈100-fold reduction in average fungal colony counts compared with the group that received PBS (Fig. 2; Table 2). Thus, despite the absence of a significant difference in average survival, the mice given mAb 2H1 had reduced fungal burden relative to those that received PBS. This result is similar to that of other investi-

Table 1. Summary of survival data

Exp.	Mice	Group	*n*	Events	Censor	Inoculum	Survival, days (mean ± SEM)	*P* value
1	A/J	Control	10	10	0	5000	4.70 ± 0.25	
		2H1	10	9	1	5000	16.5 ± 3.88	0.0001*
2	A/J	Control	10	10	0	5000	5.95 ± 0.61	
		2H1	10	9	1	5000	7.20 ± 0.83	0.1348
3	A/J	Control	30	29	1	1000	7.85 ± 0.38	
		2H1	30	29	1	1000	10.10 ± 0.83	0.0232*
4	BALB/c	Control	9	9	0	1000	6.33 ± 0.30	
		2H1	10	9	1	1000	14.10 ± 3.25	0.007*

n, No. of mice in each group. Events, no. of mice that actually died in the experiment. Censor, no. of points that were censored in the statistical analysis of the survival data; censored points correspond to animals alive at the end of the experiment. When experimental points are censored the estimate of the mean is biased against prolongation of survival. Inoculum, no. of organisms inoculated.
*Significant at the 0.05 level or better.

Table 2. Brain and lung tissue fungal burden for experiments 2, 3, and 5, in mice given PBS (control), mAb 36-65 (isotype-matched control), or mAb 2H1

			Brain tissue CFUs			Lung tissue CFUs		
Exp.	Group	*n*	Mean	SEM	*P*	Mean	SEM	*P*
2	PBS	5	5.78×10^6	2.59×10^6				
	2H1	5	7.90×10^4	4.85×10^4	0.059			
3	PBS	5	5.74×10^6	5.75×10^5				
	2H1	4	8.52×10^5	3.37×10^5	<0.001*			
5	PBS	6	2.64×10^6	2.78×10^5		2.94×10^5	2.37×10^4	
	36-65	6	1.43×10^6	5.98×10^5	0.1	2.49×10^5	1.06×10^5	0.37
	2H1	7	2.06×10^5	5.23×10^4	<0.001*	1.73×10^4	5.65×10^3	<0.001*

Lung colony-forming unit (CFU) data were obtained only in experiment 5. *n*, No. of mice in each group. *P* values were calculated by Student's *t* test for the data versus PBS control. The data for experiment 2 are shown in Fig. 2. Note that despite a difference in mean CFUs for the PBS and 2H1 groups which is almost 100-fold, the variation within the two groups results in a large variance and the calculated *P* value just misses statistical significance at the 0.05 level. In experiment 3 the 2H1 group used 5 mice. However, one mouse had a CFU count much higher than any other mouse in either the PBS or the 2H1 group, and if this single point is included it increases the 2H1 mean CFUs to 2.84×10^6, SEM to 2.00×10^6, and the calculated *P* to 0.24. Significant skewing of the mean results from inclusion of this one outrider value and hence it is not included in the table. In experiment 5, one of the mice in the 36-65 group received a nasal injection instead of an i.c. injection (the needle passed through the skull and the *C. neoformans* suspension was administered largely in the nasal cavity). This animal had few CFUs in its brain and becomes an outrider value which decreases the mean of the 36-65 group relative to the PBS group. Inclusion of this point does not result in a significant difference between the PBS and 36-65 groups and it is included in the table.

*Significant at the 0.05 level or better.

gators who have shown reduction in organ colony counts without a concomitant increase in survival for parenterally infected mice given passive antibody (32).

In experiment 3, the size of the mAb 2H1 and control groups was increased to 30 mice to clarify the statistical significance of the small survival difference observed in experiment 2. The average survival times of the PBS- and mAb 2H1-treated groups were 7.85 and 10.1 days, respectively ($P = 0.0232$). Cultures of brain tissue in a subset of 5 mice sacrificed at day 4 (not included in the survival analysis) revealed decreased *C. neoformans* colony counts in mice given mAb 2H1 relative to those given PBS i.p. (Table 2).

Experiment 4 differed from the previous three experiments in that it used BALB/c mice and mAb 2H1 was administered ≈15 hr after i.c. infection. BALB/c mice are more resistant to intravenous *C. neoformans* infection than A/J mice (55, 60), but succumbed rapidly to i.c. infection with strain 24067 (Table 1). The average survival of mAb 2H1-treated mice was twice that observed for the PBS control group ($P = 0.007$).

Experiment 5 was designed to determine the effect of mAb 2H1 on the fungal burden in brain and lung tissues relative to control groups receiving either PBS or mAb 36-65 (an IgG1 isotype-matched control ascites which does not bind cryptococcal polysaccharide). A/J mice were given 1.0 mg of mAb 2H1 or 36-65 as ascites (0.25 ml) or an equal volume of PBS i.p. 24 hr prior to i.c. infection with 1×10^3 cryptococci. There was no significant difference between the average number of *C. neoformans* colonies recovered from the tissue of mice given mAb 36-65 or PBS (Table 2 and see caption). However, mice given mAb 2H1 had an ≈10-fold reduction in the number of *C. neoformans* colonies in brain and lung tissues.

Histopathologic examination of hematoxylin- and eosin-stained tissue by light microscopy revealed a diffuse meningitis in all specimens similar to that described in another mouse model of *C. neoformans* i.c. infection (61). In one mouse (PBS group) a cystic focus of *C. neoformans* infection was noted in the brain parenchyma. In PBS control mice, immunohistochemical staining with mAb 2H1 detected numerous *C. neoformans* yeast forms throughout the meninges. In contrast, immunochemical staining revealed only rare *C. neoformans* yeast forms in the meninges of mice in the mAb 2H1 group. No staining of *C. neoformans* or brain tissues was observed with the control mAbs MOPC 21 and 2D2 (data not shown).

DISCUSSION

Administration of mAb 2H1 i.p. modified the course of i.c. *C. neoformans* infection in mice by prolonging survival and decreasing the fungal burden in brain tissue. Significant

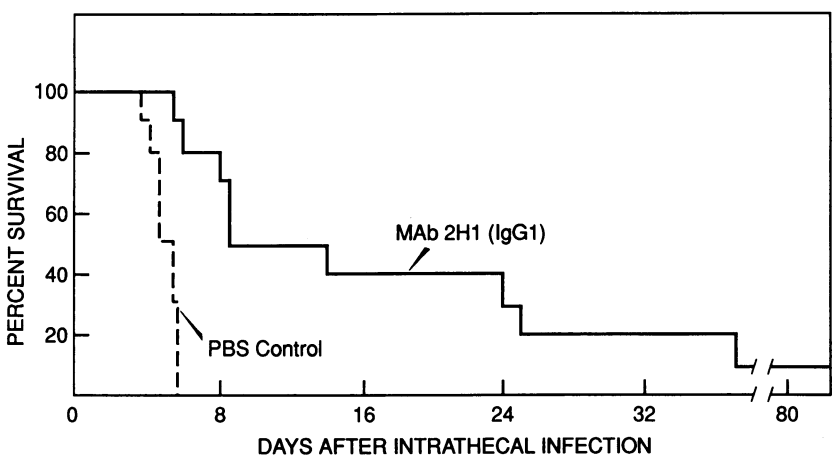

FIG. 1. Survival curves for A/J mice given either 1.0 mg of mAb 2H1 or PBS prior to i.c. infection with 5×10^3 cells of *C. neoformans* strain 24067. The curves correspond to the data for experiment 1 of Table 1.

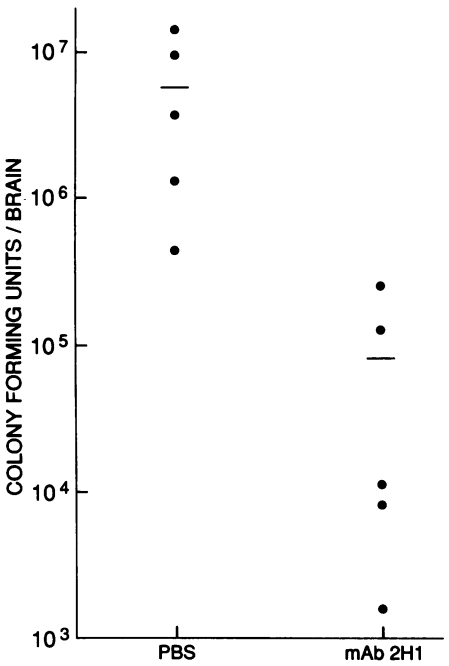

FIG. 2. Brain colony-forming units for mice given either 1.0 mg of mAb 2H1 or PBS. The five mice in each group came from a subset of the mice used in experiment 2 that were dedicated for brain colony counts.

prolongation of the average survival in mAb-treated groups was observed in three of four experiments (Experiments 1, 3, and 4). A trend toward increased survival was observed in the mAb-treated group of experiment 2 (Table 1). Reduction in the fungal burden of brain tissue was observed for the mAb 2H1-treated group in three separate experiments (Table 2). Histopathologic studies provided visual evidence of lower fungal burden in the brains of mice receiving mAb 2H1.

There was considerable interexperimental variation in the survival data. Potential causes of interexperimental variation include technical variation in the i.c. delivery of *C. neoformans* (i.e., needle trauma or dose variation), differences in animal lots such as minor differences in age or differences in nutritional status (62), and differences in the virulence of the cultures used for infecting mice. In addition, individual mice within a given lot can vary markedly in their susceptibility and their ability to mount an antibody response to *C. neoformans* (34, 35). We have observed considerable individual variation in response to i.p. infection with regard to brain, spleen, and lung tissue colony counts (unpublished data). We were not able to determine the exact cause of the interexperimental variation, or to control for it, in either the i.c. or the i.p. mouse model (30). However, despite interexperimental variation, we have consistently observed antibody-mediated protective effects in a model system where the infection and the mAb are delivered to body compartments separated by the blood–brain barrier.

The neurotropism of *C. neoformans* has been attributed to lack of opsonic activity in the central nervous system (63–66). *C. neoformans* in cerebrospinal fluid and in brain tissue are devoid of complement staining (63, 65). Complement and immunoglobulins are presumably largely excluded from the brain compartment by the blood–brain barrier. However, some serum IgG is probably able to penetrate normal rodent brains (67–69). i.c. infection with *C. neoformans* could have increased penetration of antibody into the brain compartment by disrupting the integrity of the blood–brain barrier. *C. neoformans* capsular polysaccharide can induce pathologic changes to glial cells and brain edema (70, 71), and intracerebroventricular administration of polysaccharide to rabbits results in cerebrospinal-fluid leukocytosis (72). While it is also possible that the trauma resulting from the i.c. injection could have increased antibody penetration into the brain compartment, we were not able to identify the sites of injection grossly or microscopically 4–5 days later. Irrespective of its mode of entry, the reduction of colony counts in brain tissue indicates that mAb 2H1 can mediate protective effects in the brain compartment.

The mechanism by which systemic mAb modified the course of i.c. infection is not certain. i.c. infection with complement-opsonized cryptococci in mice results in longer survival than infection with nonopsonized cryptococci, and this has been attributed to enhanced clearance of the fungus (61). Immunoperoxidase staining demonstrated that mAb 2H1 binds to the capsule of *C. neoformans* in brain sections, indicating that the mAb recognizes an epitope that is expressed *in vivo*. Protective effects could have resulted from enhanced mAb-mediated opsonic activity leading to increased cellular clearance of *C. neoformans*. i.c. infection in mice disseminates to peripheral organs (61, 73). In our mice the burden of *C. neoformans* in the lungs was ⅒th that found in the brain. mAb 2H1 also significantly reduced fungal burden in lung tissue (Table 2). Thus, mAb 2H1 may mediate protection by reducing dissemination and, possibly, reinfection of the central nervous system. Passive mAb administration could also mediate protection by enhancing the clearance of capsular polysaccharide which is associated with a variety of deleterious effects including immune paralysis (37, 38), inhibition of leukocyte migration (74) and phagocytosis (18), and the induction of pathologic changes in brain cells (70, 71).

i.c. infection in mice resembles human *C. neoformans* meningitis histologically. The i.c. infection model has been used to test antifungal drugs against *C. neoformans* (56, 57). In experiments 1 and 4, administration of mAb 2H1 resulted in prolongation of survival comparable to that observed for antifungal drugs in i.c. infection models (56, 57) and that observed when i.c. infection is done with complement-opsonized cryptococci (75). Protective effects were observed when mAb 2H1 was given before and after infection. The timing of mAb administration may not be as critical in this model because significant brain penetration by mAb may not occur until infection progresses and the blood–brain barrier becomes leaky. The prolongation in survival mediated by mAb 2H1 in the i.c. model is smaller than that observed when the infection and the mAb are both given i.p. (30). This could reflect limited mAb penetration in brain tissue, different populations of effector cells in the brain and in the peritoneum, and the fact that i.c. infection greatly enhances the virulence of *C. neoformans*.

We have observed mAb-mediated protective effects in a model system where antibody protection was not necessarily expected, because of the blood–brain barrier. Our data provide further evidence for the protective role of antibodies to *C. neoformans* infection and suggest that it is worth examining systemic and/or intrathecal serotherapy for the treatment of cryptococcal infections in humans. The observation that some AIDS patients have enhanced leakage of serum protein across the blood–brain barrier (76) suggests that antibody penetration of the brain might be significantly increased in this disease. We believe that mAb 2H1 or its derivatives are potential candidates for use in a clinical trial as an adjunct to standard antifungal therapy.

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1. Chuck, S. L. & Sande, M. A. (1989) *N. Engl. J. Med.* **321,** 794–799.
2. Zuger, A., Louie, E., Holzman, R. S., Simberkoff, M. S. & Rahal, J. J. (1986) *Ann. Intern. Med.* **104,** 234–240.
3. Eng, R. H. K., Bishburg, E. & Smith, S. M. (1986) *Am. J. Med.* **81,** 19–23.
4. Perfect, J. R., Durack, D. T. & Gallis, H. A. (1983) *Medicine* **62,** 98–109.
5. Kwon-Chung, K. J. & Bennett, J. E. (1992) in *Medical Mycology*, eds. Kwon-Chung, K. J. & Bennett, J. E. (Lea & Febiger, Philadelphia), pp. 397–446.
6. Diamond, R. D. (1990) in *Practice and Principles of Infectious Diseases*, eds. Mandell, G. L., Douglas, R. G., Jr., & Bennett, J. E. (Churchill Livingstone, New York), pp. 1980–1989.
7. Spitzer, E. D., Spitzer, S. G., Freundlich, L. F. & Casadevall, A. (1993) *Lancet* **341,** 595–596.
8. Powderly, W. G. (1992) *Clin. Infect. Dis.* **14,** S54–S59.
9. Murphy, J. A. (1991) *Annu. Rev. Microbiol.* **45,** 509–538.
10. Mody, C. H., Lipscomb, M. F., Street, N. E. & Tows, G. B. (1990) *J. Immunol.* **144,** 1472–1477.
11. Hill, J. O. (1992) *J. Exp. Med.* **175,** 1685–1695.
12. Graybill, J. R. & Ahrens, J. (1981) *J. Reticuloendothel. Soc.* **30,** 347–357.
13. Kozel, T. R., Wilson, M. A. & Murphy, J. W. (1991) *Infect. Immun.* **59,** 3101–3110.
14. Kozel, T. R., Pfrommer, G. S. T., Cuerlain, A. S., Highison, B. A. & Highison, G. J. (1988) *Rev. Infect. Dis.* **10,** S436–S439.
15. Dromer, F., Perrone, C., Barge, J., Vilde, J. L. & Yeni, P. (1989) *Clin. Exp. Immunol.* **78,** 412–417.
16. Bolanos, B. & Mitchell, T. G. (1989) *J. Med. Vet. Mycol.* **27,** 203–217.
17. Kozel, T. R. & McGaw, T. G. (1979) *Infect. Immun.* **25,** 255–261.
18. Kozel, T. R. & Gotschlich, E. (1982) *J. Immunol.* **129,** 1675–1680.
19. Kozel, T. R. & Follete, J. L. (1981) *Infect. Immun.* **31,** 978–984.
20. Diamond, R. D. (1974) *Nature (London)* **247,** 870–874.
21. Diamond, R. D. & Allison, A. C. (1976) *Infect. Immun.* **14,** 716–720.
22. Levitz, S. M., Farrell, T. P. & Maziarz, R. T. (1991) *J. Infect. Dis.* **163,** 1108–1113.
23. Nabavi, N. & Murphy, J. W. (1986) *Infect. Immun.* **51,** 556–562.
24. Miller, M. F., Mitchell, T. G., Storkus, W. J. & Dawson, J. R. (1990) *Infect. Immun.* **58,** 639–645.
25. Diamond, R. D. & Bennett, J. E. (1974) *Ann. Intern. Med.* **80,** 176–181.
26. La Mantia, L., Salmaggi, A., Tajoli, L., Cerrato, D., Lamperti, E., Nespolo, A. & Bussone, G. (1986) *J. Neurol.* **233,** 362–366.
27. Perfect, J. R., Hobbs, M. M., Granger, D. L. & Durack, D. T. (1988) *Infect. Immun.* **56,** 849–854.
28. Graybill, J. R., Hague, M. & Drutz, D. J. (1981) *Sabouraudia* **19,** 237–244.
29. Dromer, F., Charreire, J., Contrepois, A., Carbon, C. & Yeni, P. (1987) *Infect. Immun.* **55,** 749–752.
30. Mukherjee, J., Scharff, M. D. & Casadevall, A. (1992) *Infect. Immun.* **60,** 4534–4541.
31. Mukherjee, J., Casadevall, A. & Scharff, M. D. (1993) *J. Exp. Med.* **177,** in press.
32. Sanford, J. E., Lupan, D. M., Schlagetter, A. M. & Kozel, T. R. (1990) *Infect. Immun.* **58,** 1919–1923.
33. Kozel, T. R. & Cazin, J., Jr. (1972) *Infect. Immun.* **5,** 35–41.
34. Casadevall, A. & Sharff, M. D. (1991) *J. Exp. Med.* **174,** 151–160.
35. Casadevall, A., Mukherjee, J., Devi, S. J. N., Schneerson, R., Robbins, J. B. & Scharff, M. D. (1992) *J. Infect. Dis.* **65,** 1086–1093.
36. Sundstrom, J. B. & Cherniak, R. (1992) *Infect. Immun.* **60,** 4080–4087.
37. Kozel, T. R., Gulley, W. F. & Cazin, J., Jr. (1977) *Infect. Immun.* **18,** 701–707.
38. Murphy, J. W. & Cozad, G. C. (1972) *Infect. Immun.* **5,** 896–901.
39. Baker, P. J. (1990) *Infect. Immun.* **58,** 3465–3468.
40. Kwon-Chung, K. J. & Rhodes, J. C. (1986) *Infect. Immun.* **51,** 218–223.
41. Salkowski, C. D. & Balish, E. (1991) *Can. J. Microbiol.* **37,** 834–839.
42. Casadevall, A., Dobroszycki, J., Small, C. & Pirofski, L. (1992) *Am. J. Med.* **92,** 587–590.
43. Eng, R. H. K., Bishburg, E., Smith, S. M., Geller, H. & Kapila, R. (1986) *Am. J. Clin. Pathol.* **86,** 105–107.
44. Ammann, A. J., Schiffman, G., Abrams, D., Volberding, P., Ziegler, J. & Conant, M. (1984) *J. Am. Med. Assoc.* **251,** 1447–1449.
45. Lane, H. C., Masur, H., Edgar, L., Whalen, G., Rook, A. H. & Fauci, A. S. (1983) *N. Engl. J. Med.* **309,** 453–458.
46. Dromer, F., Aucouturier, P., Clauvel, J.-P., Saimot, G. & Yeni, P. (1988) *Scand. J. Infect. Dis.* **1988,** 283–285.
47. Cecil, R. L. & Blake, F. G. (1920) *J. Exp. Med.* **32,** 1–18.
48. Cole, R. (1914) *Arch. Intern. Med.* **14,** 56.
49. Flexner, S. (1913) *J. Exp. Med.* **17,** 553.
50. Shapiro, L. L. & Neal, J. B. (1925) *Arch. Neurol. Psychiatry* **13,** 174–190.
51. Littman, M. L. (1959) *Am. J. Med.* **27,** 976–998.
52. Devi, S. J. N., Schneerson, R., Egan, W., Ulrich, T. J., Bryla, D., Robbins, J. B. & Bennett, J. E. (1991) *Infect. Immun.* **59,** 3700–3707.
53. Dromer, F. & Charreire, J. (1991) *J. Infect. Dis.* **163,** 1114–1120.
54. Gordon, M. A. & Lapa, E. (1964) *J. Infect. Dis.* **114,** 373–378.
55. Rhodes, J. C., Wicker, L. S. & Urba, W. (1980) *Infect. Immun.* **29,** 494–499.
56. Allendoerfer, R., Marquis, A. J., Rinaldi, M. G. & Graybill, J. R. (1991) *Antimicrob. Agents Chemother.* **35,** 726–729.
57. Albert, M. M., Graybill, J. R. & Rinaldi, M. G. (1991) *Antimicrob. Agents Chemother.* **35,** 1721–1725.
58. Vanbreuseghem, R. (1967) *Ann. Soc. Belge Med. Trop.* **47,** 281–294.
59. Bodenhoff, J. (1969) *Acta Pathol. Microbiol. Scand.* **75,** 177–187.
60. Dromer, F., Yeni, P. & Charreire, J. (1988) *Immunogenetics* **28,** 417–424.
61. Blasi, E., Barluzzi, R., Mazzola, R., Mosci, P. & Bistoni, F. (1992) *Infect. Immun.* **60,** 3682–3688.
62. Gadebusch, H. H. & Gikas, P. W. (1963) *J. Infect. Dis.* **112,** 125–133.
63. Diamond, R. D., May, J. E., Kane, M. C., Frank, M. M. & Bennett, J. E. (1974) *J. Immunol.* **112,** 2260–2270.
64. Igel, H. & Bolande, R. P. (1966) *J. Infect. Dis.* **116,** 75–83.
65. Truelsen, K., Young, T. & Kozel, T. R. (1992) *Infect. Immun.* **60,** 3937–3939.
66. Hobbs, M. M., Perfect, J. R., Granger, D. L. & Durack, D. T. (1990) *Infect. Immun.* **58,** 2115–2119.
67. Saija, A., Princi, P., D'amico, N., De Pasquale, R. & Costa, G. (1990) *Life Sci.* **47,** 2261–2267.
68. Fabian, R. H. & Hulsebosch, C. E. (1989) *J. Neuroimmunol.* **24,** 183–189.
69. Zlokovic, B. V., Skundric, D. S., Segal, M. B., Lipovac, M. N., Mackic, J. B. & Davson, H. (1990) *Exp. Neurol.* **107,** 263–270.
70. Hirano, A., Zimmerman, H. M. & Levine, S. (1965) *Arch. Neurol.* **12,** 189–196.
71. Hirano, A., Zimmerman, H. M. & Levine, S. (1965) *J. Neuropathol. Exp. Neurol.* **24,** 386–396.
72. Bennett, J. E. & Hasenclever, H. F. (1965) *J. Immunol.* **94,** 916–920.
73. Kao, C. J. & Schwarz, J. (1957) *Am. J. Clin. Pathol.* **27,** 652–663.
74. Diamond, R. D. (1985) in *Principles and Practice of Infectious Diseases*, eds. Mandell, G. L., Douglas, R. G., Jr., & Bennett, J. E. (Wiley, New York), pp. 1460–1468.
75. Blasi, E., Mazzolla, R., Barluzzi, R., Mosci, P., Bartoli, A. & Bistoni, F. (1991) *J. Neuroimmunol.* **32,** 249–257.
76. Rhodes, R. H. (1991) *J. Neuropathol. Exp. Neurol.* **50,** 171–173.