Pathogenesis of Cryptococcus neoformans in Congenitally Immunodeficient Beige Athymic Mice

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Mortality after intravenous challenge with 10^4 Cryptococcus neoformans demonstrated that doubly immunodeficient beige athymic (bg/bg nu/nu) mice were more susceptible to systemic cryptococcosis than either bg/bg or nu/nu mice. Infected bg/bg nu/nu mice also had a shortened lifespan compared with their bg/bg nu/+ littermates. Beige athymic (bg/bg nu/nu) but not bg/bg nu/+ mice developed cryptococcal lesions in the skin, demonstrating that C. neoformans is dermatotropic in a T-cell-deficient host. Higher numbers of C. neoformans were isolated from the lungs and spleen of infected bg/bg nu/nu than bg/bg nu/+ mice as early as day 3 after challenge, indicating that in lymphoid-rich organs, T cells can alter the course of systemic cryptococcosis early in the infection. Despite extensive abscess formation in the brains of bg/bg nu/+ mice, dissemination and growth rate of C. neoformans in the brain was similar in both genotypes. The primary histopathological feature in tissues from bg/bg nu/nu mice infected with C. neoformans consisted of foci of encapsulated yeast cells with minimal to no inflammatory response. In contrast to bg/bg nu/nu mice, bg/bg nu/+ mice mounted a vigorous inflammatory response to C. neoformans that progressed from acute to chronic inflammation. Beige athymic mice are a new animal model that will be useful in clarifying the innate and acquired immune factors important in resistance to cryptococcosis.

Cryptococcus neoformans is an encapsulated yeast that causes pulmonary, central nervous system, or systemic disease in humans. A combination of innate and acquired immune responses is required to control this fungus in vivo. The high frequency of human exposure to C. neoformans in conjunction with the low incidence of cryptococcosis in the general population indicates that innate host defense mechanisms are relatively efficient in protecting against cryptococcal infection. Numerous in vitro studies have demonstrated that polymorphonuclear leukocytes (PMN), monocytes, and macrophages (Mφ) can ingest encapsulated cryptococci opsonized with either complement or anticytoticoccal antibodies (10, 23). While PMN are clearly fungicidal, Mφ are less effective at killing C. neoformans in vitro (10). This may be due to the lack of optimal in vitro conditions used to generate activated Mφ (12, 17, 23). Additionally, natural killer (NK) cells can inhibit the growth of C. neoformans in vitro (33). Despite the importance of innate immunity, the capacity of the host to mount an adequate cell-mediated immunity (CMI) response also plays an important role in resistance to cryptococcosis (8, 24). The importance of CMI in resistance to cryptococcosis has been particularly evident in patients with acquired immune deficiency syndrome (AIDS), a disease which affects T-helper lymphocytes. In AIDS patients, cryptococcosis is the fourth most common cause of life-threatening infection (9).

Both beige (bg/bg) and athymic (nu/nu) mice have been used as murine models of cryptococcosis to elucidate the in vivo role of innate and cell-mediated immunity against C. neoformans (8, 18, 19, 26, 27). Athymic mice, which have congenital dysplasia of the thymus and lack thymus-matured T cells, have impaired CMI and partially mimic the immune deficiencies observed in individuals with AIDS. Studies of cryptococcosis in athymic mice have suggested a strong role for CMI in host defense against this disease (8, 18, 26). The beige mouse has defects in the cells responsible for innate immunity—PMN, Mφ, and NK cells. The Mφ and PMN defect in beige mice is characterized by delays in both chemotaxis and phagolysosome fusion and a reduced microbicidal capacity (6), while the NK cell defect is related to their inability to lyse NK-sensitive targets (39). Additionally, some investigators have reported a reduction in inducible T-lymphocytic responses to tumor cells indicating that beige mice may also have impaired T-cell responses under some experimental conditions (40). While studies on cryptococcosis in beige mice have been used to implicate NK cells in vivo clearance of C. neoformans (19), differences in host immune response other than NK activity (e.g., PMN, Mφ, and/or T cells) may have influenced the results.

To address the impact of T-cell defects in bg/bg mice, we used doubly immunodeficient beige athymic (bg/bg nu/nu) mice and their bg/bg nu/+ littermates (phenotypically beige) to assess how combined defects in innate immunity and thymus-matured T cells affected susceptibility to cryptococcosis. Susceptibility was assessed by lethality and culturing infected organs, while histopathology was used to assess whether differences in susceptibility corresponded with altered inflammatory responses.

MATERIALS AND METHODS

Mice. Inbred germfree (GF) bg/bg nu/nu and bg/bg nu/+ N:NIH(S) III mice (7, 13, 14) between 8 and 12 weeks of age were used in this study. To assess mortality and the course of cerebral cryptococcosis, GF nu/nu and nu/+ BALB/c mice as well as GF bg/bg and bg/+ C57BL/6N mice were also used in this study. Congenitally immunodeficient mice were produced by mating homozygous (nu/nu, bg/bg, or bg/bg nu/nu) males to heterozygous (nu+, bg/+ or bg/bg nu+/+) females. All mice were obtained from the University of Wisconsin Gnotobiotic Research Laboratory (Madison, Wis.) and were maintained in accordance with National
Institutes of Health guidelines. On the day each experiment was started, mice were removed from the GF isolator and maintained in sterile cages with filter bonnets in a laminar flow cabinet.

Verification of immunodeficiencies. To confirm T-cell defects, spleen cells from bg/bg nulnu and bg/bg nul+/+ mice were assayed for their ability to respond to the T- and B-cell mitogen concanavalin A (Con A) and lipopolysaccharide (LPS) as described previously (2). In contrast to bg/bg nul+/+ mice with functional T cells, bg/bg nulnu mice did not respond to Con A. Both genotypes had strong responses to LPS. To confirm NK defects in mice homozygous for the beige gene, splenic NK activities were compared in a 4-h 

\[ ^{51} \text{Cr} \] release assay as described previously (3). Mice were also given polyinosinic-polycytidylic acid (poly(I:C)), 100 μg, 0.1 ml, intraperitoneally 24 h prior to assay to assess in vivo modulation of NK activity. To boost NK activity in vitro, 50 U of recombinant human interleukin-2 (IL-2; DuPont, Lemon, Calif.) per well was present during the entire assay period. NK data are expressed as percent cytotoxicity ± standard error of the mean (SEM) for three to six mice at an effector-to-target cell ratio of 100:1. Histology of tissues from uninfected bg/bg nulnu and bg/bg nul+/+ mice confirmed that these mice had enlarged lysosomes in their granulocytes, defects associated with the beige gene (9).

Yeast cultures and animal inoculations. An encapsulated strain of C. neoformans serotype A (strain SLHA) was maintained on Sabouraud dextrose agar (SDA). Yeast cells were transferred to Sabouraud dextrose broth and incubated at 37°C for 48 h. Cryptococci were harvested, washed three times by centrifugation (1,000 × g, 15 min), suspended in nonpyrogenic saline, counted on a hemacytometer, and adjusted to 10⁵ cells per ml. Mice were infected by injecting 0.1 ml of yeast cell suspension into a tail vein. To verify the number of viable cells, the inoculum was serially diluted in phosphate-buffered saline (PBS), plated on SDA, and incubated at 37°C for 48 h, and CFU were determined. In mortality studies, 8 to 13 mice were used per genotype, and deaths were recorded on a daily basis. Mortality studies were terminated at 60 days after intravenous (i.v.) challenge, and the number of C. neoformans CFU in the internal organs was assessed as described below. To calculate mean survival time (MST) when mice survived to day 60, each surviving mouse was arbitrarily assigned day 61. Statistical differences in MST were determined by analysis of variance.

Microbial enumeration. Mice were killed 1, 3, 7, 14, and 21 days after i.v. challenge with C. neoformans. The kidneys, liver, lungs, spleen, and brain were removed and homogenized in 5 ml of PBS. Homogenates were serially diluted in PBS and plated in duplicate on SDA, and colonies were counted after incubation for 48 h at 37°C. Data are expressed as the mean log₁₀ number of C. neoformans per gram (dry weight) of each tissue homogenate from three mice per group for each culture interval. Dry weight was determined after drying 1 ml of tissue homogenate at 60°C for 24 h. Statistical differences in C. neoformans CFU between bg/bg nulnu and bg/bg nul+/+ mice were determined by Student’s t test and analysis of variance.

Histopathology. Tissue biopsies were collected from three mice per group on days 9, 14, and 21 after i.v. challenge. After fixation for 48 h in Hollande-Bouin fixative, specimens were dehydrated through increasing concentrations of ethanol (50, 70, 80, and 95%) and embedded in glycol methacrylate (Bio-Rad Laboratories, Richmond, Calif.). Sections (2 to 2.5 μm) were cut on a JB-4 microtome (Sorvall, Newtown, Conn.) and stained with periodic acid-Schiff (PAS). Sections stained with hematoxylin and eosin were evaluated under light microscopy.

Results

Influence of athymic and beige mutations on susceptibility to cryptococcosis. The differential susceptibility of immunodeficient mice to C. neoformans is shown in Table 1. Based on mean survival, three susceptibility groups were observed. Doubly immunodeficient bg/bg nulnu mice were the most susceptible to systemic cryptococcosis. Both bg/bg and nulnu mice were of intermediate susceptibility, while bg/+ , nul+/+, and bg/bg nul+/+ mice were the most resistant to systemic cryptococcosis. When the mean survival of each genotype was compared, each susceptibility group (sensitive, intermediate, and resistant) was significantly different (P < 0.05) from the others. NK activity. To confirm NK defects in bg/bg nulnu and bg/bg nul+/+ mice, splenic NK activities from each genotype were compared in a standard 4-h 

\[ ^{51} \text{Cr} \] release assay. Additionally, two modulators of NK activity, IL-2 and poly(I:C), were used to assess their capacity to augment NK activity. In the absence of any in vivo or in vitro modulator, bg/bg nulnu and bg/bg nul+/+ mice had very low levels of splenic NK activity (2.5 ± 1.2% [SEM] and 2.3 ± 0.9%, respectively, at an effector-to-target cell ratio of 100:1) in comparison to NK-competent bg/+ controls (11.0 ± 1.2%). When IL-2 was added to the assay, significant increases (P < 0.05) in splenic NK activity were observed for both bg/bg nulnu and bg/bg nul+/+ mice (14.1 ± 4.5% and 10.4 ± 1.3%, respectively). Similar increases in splenic NK activity were observed when mice were given poly(I:C) 24 h prior to the assay (data not shown).

Pathogenesis of C. neoformans in bg/bg nulnu and bg/bg nul+/+ mice. To assess the effect of combined congenital defects in innate and cell-mediated immunity on susceptibility to cryptococcosis, doubly immunodeficient bg/bg nulnu mice and their bg/bg nul+/+ littermates were challenged i.v. with C. neoformans (Fig. 1). The numbers of C. neoformans cultured from the internal organs of bg/bg nulnu mice increased throughout the study, and the remaining mice in this challenge group died 12 to 17 days after i.v. challenge. bg/bg nul+/+ mice had significantly fewer (P < 0.05) C. neoformans CFU in the lungs and spleen than bg/bg nulnu mice as early as day 3 (e.g., 1.2 and 1.3 log₁₀ CFU, respectively). Similar increasing numbers of C. neoformans were observed in the kidneys and liver of bg/bg nulnu and bg/bg nul+/+ mice on days 1, 3, and 7 after i.v. challenge. By day 14, however, bg/bg nul+/+ mice had significantly fewer (P < 0.05) CFU in their kidneys and liver than bg/bg nulnu mice. The most dramatic difference in susceptibility of bg/bg nulnu and bg/bg nul+/+ mice to C. neoformans occurred on
day 14, when bg/bg nul+ mice had more CFU (e.g., 1.8 \( \log_{10} \) CFU in the kidneys, 2.9 \( \log_{10} \) CFU in the liver, 2.0 \( \log_{10} \) CFU in the lungs, and 3.7 \( \log_{10} \) CFU in the spleen) than bg/bg nul+/+ mice.

**Cerebral cryptococcosis.** Figure 2 shows the course of cerebral cryptococcal infection in the brains of mice with congenital immunodeficiencies in innate immunity (bg/bg, bg/bg nul+) or CMI (nul/nul) or combined defects in innate immunity and CMI (bg/bg nul/nul) as well as immunocompetent mice (nul+/+, bg/+). Similar numbers of C. neoformans CFU were cultured from the brains of bg/bg nul/nul and bg/bg nul+/+ mice throughout the study. On days 1 and 3, nul/nul mice had significantly fewer (\( P < 0.05 \)) C. neoformans CFU than bg/bg or bg/bg nul/nul mice. Despite these early differences, C. neoformans had a similar growth rate in the brains of all mice regardless of whether mice were immunodeficient or immunocompetent.

**Histopathology.** To assess how defects in CMI influence inflammatory responses and whether differences in susceptibility of bg/bg nul/nul and bg/bg nul+/+ mice to systemic cryptococcosis correlated with altered inflammatory responses, histopathology of various tissues from i.v.-challenged mice was assessed. Histology samples were collected from bg/bg nul/nul mice on days 9 and 14 and from bg/bg nul+/+ mice on days 9, 14, and 21.

**Lung.** By day 9, bg/bg nul/nul mice had developed severe lobar pneumonia. Encapsulated yeast cells, alveolar Mφ, numerous PMN, and fibrin filled the alveolar spaces (Fig. 3A) and congested the alveolar septa (Fig. 3B). By day 14 in bg/bg nul/nul mice, large foci of encapsulated yeast cells were distributed throughout the lung, and these foci evoked a vigorous inflammatory response consisting of PMN, Mφ, and fibrin. While alveolar septa of bg/bg nul/nul mice on day 14 were still congested, interstitial pneumonia was less severe than on day 9. The histopathology in the lung of bg/bg
FIG. 3. Photomicrographs of C. neoformans-infected tissues from bg/bg nu/nu and bg/bg nu/+ mice. Bars, 50 μm. (A) Lung from a bg/bg nu/nu mouse, day 9. Severe lobar pneumonia, consisting of PMN (*), fibrin (arrowhead), and encapsulated yeast cells, filled alveolar spaces and congested alveolar septa (→). Inset A1, Closeup at region near arrowhead showing fibrin deposition (→). Inset A2, Closeup at region near asterisk (*) showing numerous PMN surrounding encapsulated yeast cells. (B) Lung from bg/bg nu/nu mouse, day 9. Closeup of panel A at region indicated by →. Alveolar septa were congested with PMN, MΦ, and fibrin. (C) Lung from a bg/bg nu/+ mouse, day 9. Inflammatory foci contained encapsulated yeast cells interspersed with primarily MΦ and some PMN. Note reduced interstitial inflammation compared with panel A. Inset C1, Closeup near arrow (→) showed numerous alveolar MΦ (arrowhead) interspersed with encapsulated yeast cells. (D) Lung from a bg/bg nu/+ mouse, day 21. Inflammatory foci contained PMN (p), lymphocytes (→), MΦ (arrowhead), giant cells with ingested encapsulated yeast cells (g), and fibroblasts. (E) Brain from a bg/bg nu/nu mouse, day 9. Foci of encapsulated yeast cells with no host inflammatory response. (F) Brain from a bg/bg nu/+ mouse, day 21. Abscess consisted of a large focus of encapsulated yeast cells surrounded by primarily PMN (→), fibrin, and cellular debris. Inset F1, Closeup near arrow (→) showing PMN (p) infiltrate. (G) Meninges of a bg/bg nu/+ mouse, day 14. Encapsulated yeast cells surrounded by a few PMN and MΦ. (H) Skin from a bg/bg nu/nu mouse, day 14. Numerous encapsulated yeast cells were located in the dermis. Epidermis is indicated by *.
nul+ mice on day 9 differed dramatically from that of bg/bg nulnu mice. The inflammatory response in bg/bg nul+ mice was less severe and consisted of proportionally fewer PMN and increased numbers of Mφ surrounding large foci of encapsulated yeast cells and fibrin deposition (Fig. 3C). In bg/bg nul+ mice on day 14, foci of encapsulated yeast cells had enlarged, Mφ were the predominant inflammatory cell, and interstitial inflammation was pronounced. By day 21, interstitial pneumonia in bg/bg nul+ mice had abated, and foci of encapsulated yeast cells were surrounded by areas of both acute (PMN and fibrin) and chronic (Mφ, lymphocytes, giant cells, and collagen) inflammation (Fig. 3D).

Brain. Large foci of encapsulated yeast cells were scattered throughout the cerebrum and cerebellum of bg/bg nulnu and bg/bg nul+ mice. No cellular infiltrate was observed in or around cryptococcal foci in bg/bg nulnu and bg/bg nul+ mice (days 9 and 14; Fig. 3E). In contrast to histopathology on days 9 and 14, a strong acute inflammatory response was observed in bg/bg nul+ mice on day 21, consisting primarily of PMN, fibrin, and a few Mφ (Fig. 3F). Meningitis was also observed in bg/bg nulnu and bg/bg nul+ mice. Meningeal lesions in bg/bg nulnu mice contained encapsulated yeast cells and no cellular infiltrate on days 9 and 14, while encapsulated yeast cells surrounded by a few PMN and Mφ were observed in bg/bg nul+ mice on days 14 and 21 (Fig. 3G).

Skin. bg/bg nulnu mice began developing macroscopic skin nodules 7 days after i.v. challenge. Histopathology of the skin revealed large collections of encapsulated yeast cells in the dermis with a minimal inflammatory response consisting of Mφ (Fig. 3H). Conversely, skin lesions were not observed in bg/bg nul+ mice.

Kidney. No inflammatory response was observed around foci of encapsulated yeast cells in bg/bg nulnu mice on days 9 and 14, while minimal inflammation consisting of a few PMN and Mφ was observed surrounding cryptococcal foci in bg/bg nul+ mice on day 14. In bg/bg nul+ mice by day 21, foci of encapsulated yeast cells were surrounded by both acute and chronic inflammation. In addition, bg/bg nul+ mice (day 21) also had foci of chronic granulomatous inflammation characterized by giant cells with or without ingested yeast cells, Mφ, and some lymphocytes.

Liver. Two histopathological features were observed in the liver of bg/bg nulnu mice on days 9 and 14. Foci of encapsulated yeast cells with no host inflammatory response predominated, while abscesses that contained encapsulated yeast cells surrounded primarily by PMN and cellular debris were observed less frequently. While the latter histopathology was also observed in bg/bg nulnu mice (day 9), abscess and formation predominated. Compared with those in bg/bg nulnu mice, abscesses in bg/bg nul+ mice contained both encapsulated and nonencapsulated yeast cells and more PMN. By day 14, there was a shift from an acute to chronic inflammatory response in the liver of bg/bg nul+ mice. Chronic inflammatory foci in bg/bg nul+ mice contained primarily Mφ and nonencapsulated or degraded yeast cells. Granulomas containing Mφ, yeast cells, and giant cells with ingested organisms were observed on day 14 in bg/bg nul+ mice but were not a predominant histopathological feature until day 21.

Spleen. Two histopathological features were observed in the spleen of both bg/bg nulnu and bg/bg nul+ mice on day 9. First, large foci of encapsulated organisms were surrounded by PMN in bg/bg nulnu mice and by both PMN and Mφ in bg/bg nul+ mice. The second type of lesion, which was more common in bg/bg nul+ mice than in bg/bg nulnu mice, was characterized by small numbers of encapsulated, nonencapsulated, and degraded yeast cells interspersed with numerous PMN, some Mφ, and cellular debris. While large foci of encapsulated yeast cells still predominated in bg/bg nulnu mice on day 14, the number of abscesses had increased and PMN infiltrate predominated. In contrast, the cellular infiltrate was predominantly Mφ in bg/bg nul+ mice on day 14. Morphological forms of C. neoformans resembling hyphae were observed on day 14 in both bg/bg nulnu and bg/bg nul+ mice.

DISCUSSION

Our results show that mice with combined defects in both innate and T-cell-mediated immunity (bg/bg nulnu) have a dramatically increased susceptibility to systemic cryptococcosis. The lifespan of infected doubly immunodeficient beige athymic mice was 25 to 50% shorter than the lifespan of infected beige mice (bg/bg and bg/bg nul+) and 33% shorter than that of infected nulnu mice. Both culture data and histopathology indicated that the rapid mortality of bg/bg nulnu mice to C. neoformans was due to severe lobar pneumonia complicated by overwhelming systemic cryptococcosis, including cerebral manifestations. Furthermore, this study indicates that T cells can alter the course of cryptococcal infection at very early intervals, since bg/bg nulnu mice had more C. neoformans CFU than bg/bg nul+ mice in their spleen and lungs by day 3. The increased tissue burdens in the lungs of bg/bg nulnu mice indicated that T cells play an important role in controlling C. neoformans growth early after infection in organs rich in lymphoid cells and/or tissue Mφ. These results were unexpected, since other investigators have shown that delayed-type hypersensitivity responses to cryptococcal antigens were not detectable until 14 days after challenge with viable organisms (24, 25).

The mechanism(s) by which T cells mediated the early resistance observed in the lungs and spleen of bg/bg nul+ mice is not clear, but it has been established that T cells, either through direct cytokinetic effects (16, 22) or secretion of lymphokines that would activate other effector cells (12, 17, 33, 41), are central in resistance to cryptococcosis (11, 30). Despite low NK activity in both bg/bg nulnu and bg/bg nul+ mice, we have shown that NK cells from both genotypes were capable of responding to IL-2 and the interferon inducer poly(I:C). Since bg/bg nulnu mice lack CD4+ T cells and the cytokines produced by these cells, some of the signals that enhance the functional activities of NK cells, Mφ, and cytokotoxic T lymphocytes are missing, and this may contribute to the increased susceptibility of bg/bg nulnu mice to cryptococcosis. An analogous situation occurs in AIDS patients, who have normal levels of circulating NK cells, but the activity of NK cells is reduced due to their dependence on interleukins from CD4+ lymphocytes (35). The role of NK cells in innate immunity to C. neoformans in humans is unclear, because human NK cells require antibody to inhibit cryptococcal growth in vitro (28).

Histopathology data indicated that T cell-phagocytic cell interactions are important for production and progression of situ inflammatory responses and resistance to C. neoformans. The increased susceptibility of bg/bg nulnu mice was associated with altered inflammatory responses. First, most of the cryptococcal lesions in bg/bg nulnu mice evoked no inflammatory response at times when inflammatory responses were observed in bg/bg nul+ mice. Second, acute
inflammatory responses, when observed in bg/bg nulnu mice, were delayed in onset and consisted of fewer PMN than similar foci in bg/bg nul/+ mice. In addition, MΦ were rarely observed in inflammatory foci in bg/bg nulnu mice, whereas MΦ were observed in cryptococcal lesions from bg/bg nul/+ mice. Fourth, bg/bg nulnu mice did not develop pneumococcal pneumonia. Moreover, no fibroblast proliferation or collagen deposition was observed in cryptococcal lesions from bg/bg nulnu mice. Thus, our data indicate that the absence of thymus-matured T cells in bg/bg nulnu mice altered phagocytic infiltration and delayed induction of both acute and chronic inflammatory responses. The importance of such T cell-phagocytic cell interactions in resistance to mycotic infection has also been demonstrated by Cantorna and Balish (7), who showed that bg/bg nulnu mice also have an enhanced susceptibility to mucosal candidiasis. It has been demonstrated that T-suppressor cell circuits are induced following C. neoformans infection and mediate suppression of the delayed-type hypersensitivity response to cryptococcal antigen (31, 32). Our in vivo data suggested that the presence of T cells appears to be beneficial to the host, despite any negative effects that might be induced by the presence of T-suppressor cells.

Previous studies have associated the beige gene with increased susceptibility to cryptococcosis (19, 27). One striking observation in our studies was the relative resistance of phenotypically beige (bg/bg nul+) mice to systemic cryptococcosis compared with mice of the beige phenotype used in other studies (27). In this study, bg/bg C57BL/6N mice challenged i.v. with 104 C. neoformans had an MST of 20 days, while bg/bg nul+ N:NIH(S) III mice challenged i.v. with the same inoculum and strain of C. neoformans had an MST of 31 days. NK cell assays and histological verification of granule defects performed in this lab have confirmed that both bg/bg nul+/ N:NIH(S) III and bg/bg C57BL/6N mice have the defects associated with homozygosity for the beige gene. Similar differences in the relative susceptibility of beige C57BL/6N and C3H/Hej mice to systemic cryptococcosis were also observed by Marquis et al. (27), who reported that C3H/Hej beige mice were less susceptible than C57BL/6N beige mice. The reason for such differences in susceptibility is unclear, but is probably related to other autosomal genes that influence host resistance to infectious agents (27, 37).

Based on survival rates in inbred mice following i.v. challenge with C. neoformans, C57BL/6N mice are considered a relatively resistant mouse strain (27, 37). Fostad et al. (13) reported higher mitogenic responses to phytohemagglutinin, Con A, and LPS as well as higher sheep erythrocyte plaque-forming responses in wild-type N:NIH(S) III mice than in bg/bg C57BL/6N mice, suggesting that differences in immune responsiveness may exist between these two strains; however, no comparative data are currently available on the resistance (or susceptibility) of wild-type N:NIH(S) III and C57BL/6N mice to systemic cryptococcosis.

A striking observation in this study was the extensive abscess formation in the brains of bg/bg nul+ mice on day 21. Typically, investigators have reported that little or no inflammatory response is evident in the brains of C. neoformans-infected mice (26, 27, 29) regardless of whether the mice were immunodeficient, immunocompetent, or null. The lack of inflammatory responses in the brains of mice, particularly immunocompetent mice, may be related to the high challenge inoculum (105 and 106 organisms) used in the latter studies (26, 27, 29), which can overwhelm host defense mechanisms. Despite abscess formation in bg/bg nul+ mice, our culture data indicate that the growth of C. neoformans was similar in the brains of immunodeficient and immunocompetent mice. This apparent lack of control of C. neoformans growth in the brain may be related to a delayed induction of inflammatory responses and the presence of inflammatory cells only at the periphery of very large foci of encapsulated yeast cells.

Cutaneous manifestations of cryptococcosis occur in 10 to 15% of the cases of disseminated disease in humans (36), and recent findings suggest that cutaneous cryptococcosis may not be as uncommon as previously thought. Immunosuppressed individuals appear to be at particular risk, including renal transplant, cancer, and AIDS patients (5, 20, 38). In this study, skin lesions were only observed in bg/bg nulnu mice, suggesting that C. neoformans is dermatotropic in a T-cell-deficient host.

Gnotobiotic immunodeficient mice are excellent animal models with which to study pathogenesis of and immunity to cryptococcosis. The lifespan of conventionally reared immunodeficient animals is often shortened (1, 21), and nude mice were highly susceptible to chronic wasting disease, infection with viruses, and Pneumocystis carinii pneumonia (1, 21, 34, 42). In addition, a wide range of pathogenic agents can alter host immunity and complicate research with immunodeficient mice (15, 34). This is important when one considers that infection with C. neoformans is immunosuppressive, both specifically for cryptococcal antigens (32) and nonspecifically for unrelated antigens (4). It is possible that the C. neoformans-induced immunosuppression could increase the susceptibility of immunodeficient mice to secondary infections, which would alter both mortality and disease expression. The gnotobiotic mice used in this study were free of secondary bacterial infections as assessed by both culture data and histopathology. Beige athymic mice are a new murine model that will be useful in clarifying the components of innate and acquired immunity involved in resistance to systemic cryptococcosis.

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LITERATURE CITED