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Fungal-Strain-Dependent Alterations in the Time Course and Mortality of Chronic Murine Pulmonary Blastomycosis

STEPHEN A. MOSER,[1]* PAUL J. KOKER,[2]† AND JAMES E. WILLIAMS[2]

Department of Pathology, The Jewish Hospital at Washington University Medical Center, and Washington University School of Medicine, Saint Louis, Missouri 63110,[1] and Department of Pathology, University of Wisconsin Medical School at Mount Sinai Medical Center, Milwaukee, Wisconsin 53201[2]

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Intratracheal injection of 10^4 conidia of *Blastomyces dermatitidis* strain M1-A into mice was shown in previous work to induce chronic pulmonary and disseminated infection with histopathologic features of chronic human blastomycosis. Furthermore, 10-fold variations in inoculum density produced marked changes in mean survival times (MSTs), i.e., 32 days at 10^6, 36 days at 10^5, 97 days at 10^4, and 172 days at 10^3. A second strain (M1-B) failed to induce death in this model. To assess fungal-strain-dependent virulence, we extended these previous studies to 11 additional human isolates. Groups of male BALB/cByJ mice (6 to 8 weeks old) were injected intratracheally with 10^4 conidia from each of the 13 strains; the mice were weighed weekly and monitored for mortality, and their lungs were examined histopathologically. Infection rate and mortality were 100% in all groups except for the M1-B inoculated mice. For strains M1-B and M1-A, MST and mortality curves were not significantly different from those observed in our previously reported experiments. Four different survival patterns were noted in infected mice. The shortest MSTs were produced by strains M2-E, M2-B, M2-K, M2-H, and M2-A (24, 26, 26, 27, and 31 days, respectively), and the longest MST was seen in animal groups inoculated with strains M2-J and M2-G (130 and 134 days, respectively). Strains M2-I, M2-F, and M2-D produced intermediate MSTs of 65, 79, and 80 days, respectively. The 107-day MST induced by M1-A did not differ from strain M2-C-induced MST but differed significantly from the MST produced by the other strains. Pulmonary histopathology was similar in all animals dying with blastomycosis. The wide spectrum in survival times was not related to differences in clinical status of the patient from whom the isolate had been obtained, to fungal inoculum viability, or to individual mouse weight at inoculation. Strain-dependent virulence factors present in these fungal isolates alter the disease course in inbred mice in a fashion similar to that induced by 10-fold inoculum variation of strain M1-A conidia. These 13 isolates of *B. dermatitidis*, 1 avirulent and 12 with differing levels of virulence, provide tools for future studies into the nature of fungal virulence determinants in chronic blastomycosis.

Since the first report of human blastomycosis (10), many of the strains of *Blastomyces dermatitidis* used to produce animal models have not been preserved (1–3, 14, 20). A few strains used in murine studies of blastomycosis have been deposited with the American Type Culture Collection. These include environmental isolates KL-1 (ATCC 26198) (5, 8, 11) and MCG-1 (ATCC 32090) (8) and human isolates GA-1 (ATCC 26197) (5, 8, 11) and SCB-2 (ATCC 26199) (5, 7, 11).

Murine models of blastomycosis are fundamental to investigations of pathogenic and immune mechanisms in this chronic mycotic infection. Murine infection has been produced by intravenous (7) and intraperitoneal (16) inoculation of yeast-phase organisms. Studies using models in which infection was induced by yeast-phase *B. dermatitidis* have shown differences in pathogenicity related to route of inoculation (16), inoculum size (7, 11), fungal strain (6, 11), and mouse strain (17). Reports of variance in conidial pathogenicity related to conidial inocula are limited to *B. dermatitidis* strains KL-1 and 181-2 (D. J. Drutz, P. L. DeMarsh, and C. L. Frey. Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, F4, p. 365).

The route of primary infection in naturally acquired blastomycosis of humans, dogs, and other mammals is generally accepted to be inhalation of infective particles from the environment. Both intranasal yeast (11) and conidial inoculation (Drutz et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1985) have been used to produce murine models of primary pulmonary infection. Conidia have been shown to produce a chronic infection (22; Drutz et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1985), whereas yeasts produce a rapidly fatal infection even at low inocula (11). With *B. dermatitidis* strains 181-2 and KL-1 (Drutz et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1985), marked differences in mortality were observed with intranasal inoculation conidia as opposed to similar mortalities produced by yeast forms inoculated by the same route. Intranasal inoculation of 5×10^4 conidia of 181-2 produced 50% mortality at 140 days, while KL-1 did not produce mortality in that time frame. In contrast, the yeast form of both strains was equally virulent, killing all mice within 49 days after intranasal inoculation with 10^2 organisms.

In our recently reported murine model of chronic pulmonary blastomycosis induced by intratracheal injection of purified *B. dermatitidis* conidia (22), we used both a virulent (M1-A) and an avirulent (M1-B) strain obtained from cases of human infection which had not previously been assessed for pathogenicity in mice. Conidia of strain M1-B failed to produce infection, assessed by weight loss or death within a 329-day observation period. Disease produced by 10^4 conidia of strain M1-A had a mean incubation period of 55 days and 50% mortality at 92 days. Pulmonary histopathology was similar to that reported in naturally acquired human infection

* Corresponding author.
† Present address: Boehringer Ingelheim, P.O. Box 368, Ridgefield, CT 06877.

(19, 21). In contrast, mice inoculated intranasally with yeasts had pulmonary histopathologic responses strikingly different from those seen in human disease (11). Histopathologic features of pulmonary infection induced by intranasal installation of conidia have not been reported.

The current study was undertaken to determine whether 11 additional human isolates produced conidia which might induce different mortality patterns in our murine model of pulmonary blastomycosis. Characterized *B. dermatitidis* strains with differing degrees of pathogenic potential should provide useful probes for investigations into the nature of pathogenic mechanisms in blastomycosis.

(A preliminary report of these findings was presented at the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 28 September to 1 October 1986 [S. A. Moser, P. J. Koker, and J. E. Williams, Program Abstr. 26th ICAAC, abstr. no. 796, 1986].)

MATERIALS AND METHODS

Animals. BALB/cByJ male mice (Jackson Laboratories, Bar Harbor, Maine) 6 to 8 weeks old were used in these experiments. Control and inoculated animals were housed in the same room and provided food and water ad libitum.

Organisms. Eleven strains of *B. dermatitidis* (M1-A, M2-B, M2-C, M2-D, M2-E, M2-F, M2-G, M2-H, M2-I, M2-J, and M2-K) were isolated from sporadic human cases with pulmonary or disseminated blastomycosis who had been tested for the presence of antibodies to *B. dermatitidis* by double immunodiffusion (Nolan Biologicals, Atlanta, Ga.). Stock cultures were obtained as direct subcultures of the primary isolates and were transferred once to freezing agar (15) and maintained at −70°C until use (approximately 6 to 15 months). Two additional strains of human origin (M2-A and M1-B) were obtained from the Centers for Disease Control and treated in a fashion similar to our clinical isolates. There was no record, however, of the number of subcultures to which these organisms were subjected prior to our receiving them. Strains M1-A and M1-B correspond to the virulent and avirulent strains, respectively, described by Williams et al. (23).

Isolation and preparation of conidial suspensions. Details of these procedures have been reported previously (22). Briefly, conidia were prepared from Pine-Drouhet agar (18) plates incubated at 30°C in ambient air for 2 to 3 weeks by pipetting sterile, nonpyrogenic 0.15 M saline (NPNS) (Travenol Laboratories, Deerfield, Ill.) onto the plates and rubbing the surface with a Pasteur pipette bent 45° at the tip (i.e., hockey stick). The conidia-containing fluid from three or four plates was aspirated and pooled in a sterile 50-ml conical plastic centrifuge tube (Falcon Division, Becton Dickinson, Oxnard, Calif.). After gentle agitation by alternate aspiration and expurgation through a 22-gauge needle with a 10-ml syringe, the fluid was centrifuged at 2,000 × *g* for 10 min at room temperature. Conidia were suspended in sterile NPNS and separated from hyphal segments by filtration through a 5-μm pore-size polycarbonate filter (Nuclepore Corp., Pleasanton, Calif.). The conidia which passed through the filter were washed twice and resuspended in NPNS. Total conidial counts were determined on a hemacytometer and contained ≤7% hyphal fragments (range, 3 to 7%).

Conidial viability. (i) Germination. Conidia were incubated in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) for 24 h at 30°C in ambient air. The germinating conidia were counted on an inverted microscope (Nikon Inc., Instrument Division, Garden City, N.Y.) at a magnification of 300×, and results were expressed as the percentage of total conidia which had germinated.

(ii) CFU. Based on the hemacytometer count, 0.1 ml of appropriate dilutions of conidial suspensions was plated onto either Sabouraud dextrose agar with chloramphenicol (SDA-C) or Kelley agar (KA) (15) and incubated at 30 and 37°C, respectively, in ambient air. Colonies were counted after 7 to 10 days of incubation, and the average of triplicate counts was expressed as a percentage of CFU per total hemacytometer conidial count.

Intratracheal inoculation. After being weighed, mice were anesthetized by intraperitoneal injection with sodium pentobarbital (65 mg/kg; Abbott Laboratories, North Chicago, Ill.) and secured, head up, with rubber bands onto an operating board inclined at 45°. Lidocaine hydrochloride jelly (2%; Astra Pharmaceutical Products, Inc., Worcester, Mass.) was applied to the neck operative site to prevent postoperative self-mutilation. A midline incision was made in the skin from the level of the hyoid bone to the manubrium, exposing the salivary gland. The two salivary gland lobes were then displaced laterally, exposing the trachea. A 27-gauge needle was inserted bevel up into the tracheal lumen, and 0.05 ml of the appropriate conidial suspension was injected in a caudal direction. Controls were inoculated with 0.05 ml of NPNS. The salivary gland was repositioned over the trachea, and the skin was closed with metal clips (Clay Adams, Parsippany, N.J.).

Experimental design. Experimental groups of 10 to 12 mice were inoculated with 10^4 conidia of each *B. dermatitidis* strain in NPNS or with NPNS alone. Both inoculated and control groups were followed to determine the time course of infection, weight change, and mortality by observing and weighing at weekly intervals until the mice expired or were sacrificed. Gross necropsy was performed on all animals which expired during the observation period. In addition, histopathologic examination (i.e., with Formalin fixation, paraffin embedding, and hematoxylin and eosin plus periodic acid-Schiff staining) was performed on lung specimens of dead animals which had not undergone severe autolysis. Animals which survived for 285 days were sacrificed and subjected to complete necropsy with histopathologic examination.

Statistics. All data are expressed as the mean with the standard error of the mean used as the index of dispersion. Comparisons of conidial viability data were performed by pooled variance *t* test after establishing equality of variance with an *F* test (RS/1, BBN Research Systems, Cambridge, Mass.). Multiple comparisons of group survival data were performed with the multiple range test (9) (SAS Institute Inc., Cary, N.C.). The starting weights and mortality were assessed by the rank correlation coefficient of Spearman's rho (RS/1). Unless otherwise specified, a significant difference was defined as a P value of <0.05.

RESULTS

Conidial viability. Mean conidial viability assessed by germination was 86 ± 1.8%, with a range of 76 to 94%. Mean CFU-determined viability was 82 ± 4.4%, with a range of 46 to 98%, on KA and 65 ± 2.9%, with a range of 55 to 89%, on SDA-C.

Strain-dependent alterations in weight change. Mean group weight change curves differed noticeably in a strain-dependent fashion. The strain M1-B-inoculated group (Fig. 1) was not different from the NPNS-inoculated control group (data

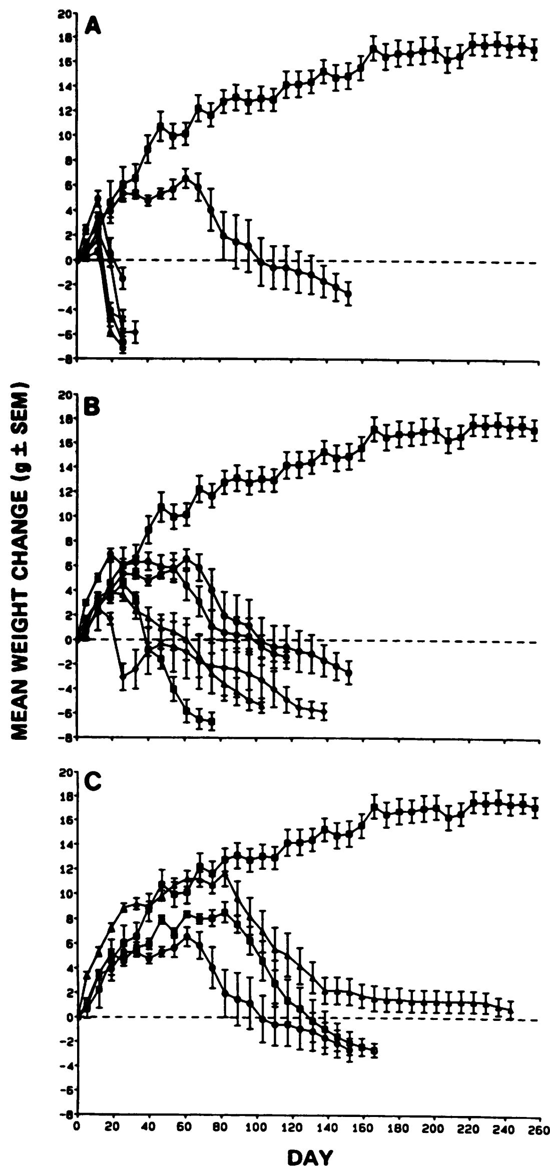

FIG. 1. Weight change curves in groups of mice inoculated with 13 strains of *B. dermatitidis* conidia. Alterations in group mean weight induced by strain M1-B (■) and strain M1-A (●) are displayed in each panel for comparison. Panels A, B, and C display strain-dependent weight change curves according to group-related similarities in mortality by Duncan multiple range testing ($P < 0.05$). (A) Following a brief initial increase, a rapid, tightly clustered decline in group mean weight is apparent for groups inoculated with strains M2-E (▽), M2-H (□), M2-K (○), M2-A (◇), and M2-B (△). (B) Curves for strains M2-I (□), M2-C (○), M2-F (◇), and M2-D (△) peak and decline prior to that for strain M1-A. (C) Strain M2-G (□) and strain M2-J (△) groups continued to gain weight after the strain M1-A-inoculated group mean weight began to fall.

not shown). Both of these groups, rapidly at first and then gradually, gained weight until 285 days postinoculation, the date of sacrifice. In each of the other 12 groups, weight change curves were markedly different from those for both NPNS- and strain M1-B-inoculated groups. Initially, weight gain in these groups mimicked the early weight gain in NPNS control and M1-B-inoculated groups; however, in each infected group the weight gain trend reversed and fell at rates which varied greatly. Specifically, these reversal points were 19 days for M2-A, M2-B, M2-E, M2-F, M2-H, and M2-K; 33 days for M2-D and M2-I; 47 days for M2-C; 68 days for M1-A; and 89 days for M2-G and M2-J. In each group, decline in mean weight preceded the onset of infection-related mortality.

Strain-dependent alterations in mortality. All mice inoculated with strain M1-B survived to the end of the 285-day observation period. The mean survival time (MST) in groups of mice inoculated with conidia from the remaining 12 strains of *B. dermatitidis* ranged from 24 to 134 days (Table 1). Analysis of group MST revealed four distinct mortality patterns (Table 1 and Fig. 2). The longest surviving groups were those inoculated with strains M2-J and M2-G (group A). Shortest survival times were produced by strains M2-E, M2-B, M2-K, M2-H, and M2-A (group D). Strains M2-I, M2-D, and M2-F (group C) induced mortality in a time frame intermediate between groups A and D. Strain M1-A (group B) produced an infection with a mortality curve not significantly different from that produced by strain M2-C, but significantly different from the mortality curve produced by the remaining 11 strains. M2-C produced a chronic infection with a mortality curve which was not significantly different from those for either group B or group C although it differed significantly from both group A and group D curves.

Characteristics related to the source of the isolate and the nature of the human disease were evaluated to determine whether they might be related to the alterations in mortality in groups of experimental animals (Table 2). Human patients ranged from 12 to 53 years old (mean, 35.4), with a male/female ratio of 1.6:1. All patients had chronic pulmonary or disseminated blastomycosis, and an A-precipitin was detected in 10 of 13 (77%) at the time of diagnosis. Although the number of patients in a group was small, there did not appear to be any correlation between the induced murine mortality curves and the features of the human infection from which the isolates were obtained.

Furthermore, there was no correlation between conidial viability as determined by CFU on either SDA-C or KA or by germination (Table 3), and virulence for mice. Moreover, statistical analysis of conidial viability in Duncan groups C and D, the only groups with large enough numbers to be compared, revealed no difference in inoculum viability regardless of the method used, i.e., germination or CFU, in these groups which had significantly different MSTs.

Although the mean starting weights of animals ranged from 20.7 ± 0.5 g to 26.3 ± 0.6 g (Table 3), there was no significant variation in the mean weights of groups of mice inoculated with strains M1-B, M2-J, M1-A, M2-D, M2-F, M2-I, M2-A, M2-H, M2-K, M2-B, or M2-E. Mean group

TABLE 1. Duncan groupings

Strain	MST (days)	*n*	Duncan group
M2-G	134	12	A
M2-J	130	10	A
M1-A	107	11	B
M2-C	88	11	C, B
M2-D	80	12	C
M2-F	79	12	C
M2-I	65	11	C
M2-A	31	11	D
M2-H	27	12	D
M2-K	26	12	D
M2-B	26	12	D
M2-E	24	11	D

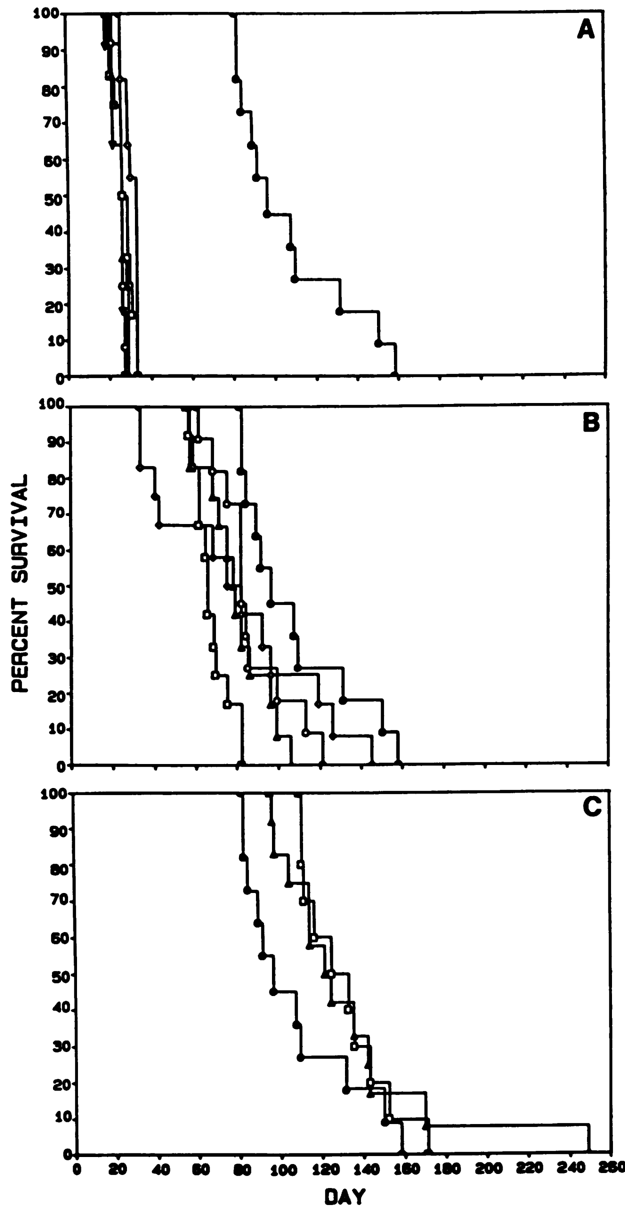

FIG. 2. Mortality curves in groups of mice inoculated with 13 strains of *B. dermatitidis* conidia. Mortality induced by strain M1-A (●) is displayed in each panel for comparison. Panels A, B, and C display mortality curves grouped by Duncan multiple range testing ($P < 0.05$). (A) Strain M2-A (◇), strain M2-B (△), strain M2-E (▽), strain M2-H (□), and strain M2-K (○) each produced rapid mortality. (B) Mortality curves of strain M2-C (○), strain M2-D (△), M2-F (◇), and strain M2-I (□) were intermediate but more rapid than that of strain M1-A. (C) Strain M2-G (□) and strain M2-J (△) yielded longer survival than strain M1-A.

weight at inoculation for mice injected with strains M2-G and M2-C was similar but lower than the other 11 animal groups. Rank correlation analysis of the relationship between individual animal weight at the time of inoculation and survival within Duncan groups revealed no significant deviation from zero with Spearman's rho values of 0.187 (n = 21), 0.210 (n = 9), −0.178 (n = 44), and −0.075 (n = 56) for groups A, B, C, and D, respectively.

Histopathology. No gross pathologic changes were observed in NPNS- or strain M1-B-inoculated mice; however, gross morphologic changes were evident in both lungs of all animals which expired during the experiments, i.e., those inoculated with strains M2-G, M2-J, M1-A, M2-C, M2-D, M2-F, M2-I, M2-A, M2-H, M2-K, M2-B, and M2-E. Usually one lung was more markedly involved than the other, with large foci of disease being distributed equally between the right and left lung. Infected foci were firm, sharply demarcated, yellow-white nodules with yellow-white granular sectioned surfaces.

Histopathologic features of infected foci showed changes similar to those reported for murine (22) and human (21) disease. Pyogranulomatous inflammation was the most common tissue reaction observed. Pyogranulomas had a central area of caseouslike necrosis comprising eosinophilic granular or amorphous material admixed with nuclear debris of inflammatory cells and yeast. Surrounding the necrotic area was an infiltrate composed of admixed polymorphonuclear leukocytes, macrophages, and giant cells. Budding and nonbudding yeast forms were internalized within both macrophages and giant cells. The outermost zone of the pyogranulomas was of irregular thickness and contained admixed clusters of plasma cells and lymphocytes. Thin bands of proliferating fibroblasts and fibrous tissue were present in larger pyogranulomas.

In addition to pyogranulomas, nodular foci of blastomy-

TABLE 2. Patient data summary for *B. dermatitidis* strains

Duncan group	Patient strain	Sex	Age (yr)	Source	Disease state[a]	Serology (double immunodiffusion)
	M1-B	F	40	Sputum	Pul, Dis	Negative
A	M2-J	M	?	Sputum	Pul	A band$^+$
A	M2-G	F	21	Lung	Pul	A band$^+$
B	M1-A	M	34	Pus-Osteo	Dis	A band$^+$
C, B	M2-C	M	33	Bronchial wash	Pul	A band$^+$
C	M2-D	M	50	Lung	Pul	A band$^+$
C	M2-F	M	31	Sputum	Pul	Negative
C	M2-I	F	46	Sputum	Pul, Dis	A band$^+$
D	M2-A	F	?	Sputum	Pul, Dis	A band$^+$
D	M2-H	M	12	Brain	Dis	A band$^+$
D	M2-K	M	38	Sputum	Pul, Dis	A band$^+$
D	M2-B	M	53	Lung	Pul	Negative
D	M2-E	F	31	Sputum	Pul	A band$^+$

[a] Pul, Pulmonary; dis, disseminated.

TABLE 3. Conidial viability of fungal strains

Fungal strain	Mice (mean wt, g ± SEM)	Conidial viability			Duncan group
		% Germination	KA (CFU)	SDA-C (CFU)	
M1-B	25.5 ± 0.6	92	82	67	
M2-G	21.2 ± 0.4	89	87	67	A
M2-J	24.8 ± 0.5	82	ND[a]	77	A
M1-A	25.5 ± 0.6	77	98	89	B
M2-C	20.7 ± 0.5	92	85	57	C, B
M2-D	25.6 ± 0.3	94	98	63	C
M2-F	25.4 ± 0.6	87	96	68	C
M2-I	25.2 ± 0.9	90	78	55	C
M2-A	26.0 ± 0.6	76	80	72	D
M2-H	26.2 ± 0.6	91	82	70	D
M2-K	23.6 ± 1.0	90	76	50	D
M2-B	26.3 ± 0.6	80	46	58	D
M2-E	23.9 ± 0.5	78	72	56	D

[a] ND, Not done.

cotic pneumonia were present. In these foci, alveolar walls were preserved and adjacent alveoli were filled by an infiltrate of polymorphonuclear leukocytes admixed with macrophages which occasionally contained phagocytized yeast forms. Necrosis and fibrosis were typically absent.

Enlarged peribronchial and mediastinal lymph nodes often contained nodules composed of yeast cells internalized within macrophages admixed with polymorphonuclear lymphocytes; however, giant cells and fibrosis were conspicuously absent.

Histopathologic features were similar in groups of animals which had similar MSTs regardless of whether altered survival was related to strain variance or inoculum variance. Pyogranulomatous inflammation was present in all animals and was the predominant inflammatory response in groups of animals with MSTs of greater than 36 days. Blastomycotic pneumonia was also present in all infected animals but, in contradistinction, formed the predominant inflammatory response in groups with an MST of less than 36 days.

DISCUSSION

We examined the potential of 13 clinical isolates of *B. dermatitidis* to produce chronic murine pulmonary blastomycosis following intratracheal inoculation of conidia. These studies confirmed both the inability of 10^4 conidia of *B. dermatitidis* strain M1-B to induce disease in this model and the reproducibility of the time course and mortality of the infection induced by inoculation with 10^4 conidia of *B. dermatitidis* strain M1-A. In addition, we have characterized the time course and mortality of infections produced by 10^4 conidia from 11 additional strains of *B. dermatitidis*.

The time course and mortality of pulmonary blastomycosis induced by conidia from these 11 strains of *B. dermatitidis* at a fixed inoculum were similar in range to the mortality induced by alterations in inoculum size, as reported previously (23), when 10^3, 10^4, 10^5, and 10^6 conidia of *B. dermatitidis* strain M1-A were used. Analysis of survival data from these strain variation studies shows that Duncan group D survival curves, those with the shortest MSTs, cannot be distinguished from survival curves produced by inocula of 10^5 or 10^6 conidia in previously reported dose-response experiments with strain M1-A (22). Duncan group A survival time is indistinguishable from the 10^3 dose of strain M1-A. In addition, strains M2-C and M1-A which compose Duncan group B, produce mortality curves which are not different from those reported for a 10^4 inoculum of M1-A (data not shown).

Histopathologic features of the infections produced by these 11 additional strains were also similar to those produced by variation in inoculum size of strain M1-A (22), with pyogranulomata being predominant in infected animals which survived longer than 36 days and blastomycotic pneumonia being the predominant inflammatory response in animals which died earlier than 36 days. Since we used syngeneic male mice in both this and our previous studies, these observations suggest that alterations in the time course of infection are due to differences in fungal isolates as well as to differences in inoculum size of a single fungal strain.

To assess other variables which might be related to the variance in strain-dependent pathogenesis, we gathered available clinical data about the patients from which each of our 13 isolates had been obtained (Table 2). These data were compared with the survival statistics and Duncan groupings to expose factors related to the mouse virulence expressed by individual isolates. No correlation was found between patient age, sex, or clinical form of disease and the fungal-strain-related alteration in murine infection. We recognize, however, that the number of patients was small and did not allow a valid statistical comparison of these parameters. It is interesting that 77% of these patients had antibody to the A antigen of *B. dermatitidis* by double immunodiffusion at the time of diagnosis. This proportion of patients is consistent with a sensitivity of 79% (12), 70% (23), and 64% (13) previously reported for chronic human blastomycosis.

Alterations in mouse mortality induced by intranasal inoculation of yeast-phase *B. dermatitidis* have been attributed to differences in mouse strain (17), route of inoculation (16), and weight and age (4). We used a single mouse strain of uniform sex and age to minimize host-related variables. However, we noted that within the 140 mice, all 6 to 8 weeks old, used in this study there was considerable variance in individual animal weight (range, 17.4 to 29.5 g). Whether the mice were assessed as groups inoculated with individual fungal strains or as individual animals within groups, we found no relationship between weight at the time of inoculation and MST. This finding is in contrast to the direct correlation between weight at inoculation and survival time reported in murine pulmonary blastomycosis induced by intranasal inoculation of yeasts (4).

We assessed conidial viability with a germination assay and two CFU assay systems, one to quantify mold growth and another for yeast recovery. There was no apparent relationship between conidial viability as assessed by any method and the MST of groups which developed blastomycosis (Table 3). Furthermore, there was no significant difference between the mean percentage of viable conidia in the two largest Duncan groups (C and D), in contradistinction to the distinct differences in group MST.

The 11 isolates of *B. dermatitidis* we studied and reported here may be added to the four strains (M1-B, M1-A, 181-2, and KL-1) which have previously been studied in conidially induced blastomycosis in animal models. In addition, our strains have been placed into groups in relationship to their ability to induce mortality and to induce varying mortality curves at a fixed inoculum dose in this model of chronic murine pulmonary blastomycosis. In naturally acquired infections, it is uncertain whether variation in inoculum size or strain differences or both are related to development of the varied clinical forms described for this infection. These 12 pathogenic and one avirulent strain will be valuable probes

for future studies of fungal factors related to pathogenesis of conidia-induced chronic pulmonary infections in mice.

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