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Susceptibility Differences of Inbred Strains of Mice to Blastomycosis

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The susceptibility of inbred strains of mice to pulmonary blastomycosis was studied to derive information relevant to host resistance and genetic background. Initial studies with eight strains with various *H-2* backgrounds revealed the C3H/HeJ strain to be highly susceptible and DBA/1J mice to be resistant. These observations were confirmed with various challenge inocula. These differences were not dependent on the size of the challenge, the strain of *Blastomyces dermatitidis*, host age, or ability of the challenge to penetrate to the lower airways. Differences between the susceptible and resistant strains in lymphocyte proliferation in vitro and delayed-type hypersensitivity in vivo after nonlethal subcutaneous infection were not demonstrated; the susceptible strain made a significantly greater antibody response to blastomyces antigens as determined by an enzyme-linked immunosorbent assay. The resistance of the C3H/HeN strain of mice, which differs from the C3H/HeJ in sensitivity to lipopolysaccharide and lacks the macrophage cytotoxicity defect of the latter, suggests that the susceptibility of C3H/HeJ mice is not related to their C3H background or the *H-2* locus. As the A/HeJ strain, which also has a macrophage cytotoxicity defect, was found in this study to be the second most susceptible strain, this also suggests macrophages as the subject for further study with respect to the mechanism of genetic resistance to this infection.

Epidemiological information concerning the endemic systemic mycoses of the Americas indicates that progressive disease occurs only in a small percentage of healthy individuals who become infected (19). Some information concerning host factors which could influence this outcome is available; for example, in coccidioidomycosis, the influence of race on outcome has long been known (20). Other genetic factors may influence the outcome of coccidioidal infection, such as blood group and HLA type (20). The availability of a model system to study host genetic influences on outcome of fungal infection would be desirable. Whereas the susceptibility of various inbred mouse strains to several infectious agents has been described, information concerning the mycoses is minimal. This information could also be illuminating, by comparing susceptible and resistant strains, in understanding the mechanism of defenses against mycotic infection.

Using a murine model of pulmonary blastomycosis, we indicated in a preliminary report (P. A. Morozumi and D. A. Stevens, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, F63, p. 373) that there are consistent, reproducible differences in susceptibility of murine strains that were independent of challenge size. We report here our confirmation of this observation, and extend these findings to studies of the effect of different *Blastomyces dermatitidis* isolates, host age, possible mechanical factors in the respiratory tree, and immune response and, by study of two closely related histocompatible strains, the effect of a gene locus in the susceptible strain.

MATERIALS AND METHODS

Mice. Healthy, 7-week-old male mice of eight inbred strains were used in the initial strain survey experiments. C3H SW/Sn, C3H/HeJ, A/HeJ, DBA/1J, DBA/2J, C57BL/10J, and SJL/J mice were purchased from the Jackson Laboratories, Bar Harbor, Maine. BALB/c/St mice were obtained from the Leonell C. Strong Research Foundation, San Diego, Calif. These strains were selected to include several *H-2* types (Table 1) (10). Subsequent experiments used male mice 4 and 10 weeks of age. C3H/HeN mice were obtained from the National Institutes of Health, Bethesda, Md. Mice were fed laboratory mouse chow (Wayne Laboratory Blox; Allied Mills, Chicago, Ill.) and sterile water ad libitum.

B. dermatitidis. The fungus used in the strain survey experiments was an isolate of *B. dermatitidis* ATCC 26199 which had been transferred extensively in the laboratory. This yeast was found to be attenuated in virulence for mice after weekly transfer on

TABLE 1. *H-2 type of strains in this study*[a]

Strain	*H-2* type
C3H/HeJ	*k*
A/HeJ	*a*
DBA/2J	*d*
C3H SW/Sn	*b*
BALB/c	*d*
SJL/J	*s*
C57BL/10J	*b*
C3H/HeN	*k*
DBA/1J	*g*

[a] The strains are ranked according to their relative resistance to pulmonary blastomycosis, starting with the most susceptible, in several comparisons described in the text.

heart infusion agar slants at 37°C for approximately 1 year (C. Brass, C. M. Volkmann, H. P. Klein, D. E. Philpott, C. Halde, and D. A. Stevens, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, F33, p. 325). The parent virulent strain was also used in several experiments. Replicate cultures of both strains were grown in the yeast phase at 37°C on heart infusion agar slants and stored at 4°C until use. A fresh culture was prepared from the stored slants before each experiment.

Preparation of yeast for intranasal challenge. Yeast cells for intranasal inoculation were prepared as previously described (12). Briefly, a synthetic medium (13) was seeded with yeast cells from the refrigerated slants and incubated at 37°C on a gyratory shaker (200 rpm) for 72 to 96 h. The cells were passaged once in broth to establish log-phase growth (72 h). Log-phase yeast cells were transferred to sheep blood agar plates and grown at 37°C for 72 h. Yeast cells were harvested with a wire loop, washed three times with sterile physiological saline, and counted in a hemacytometer. Before use, dilutions of the suspension used as the inoculum were placed on blood agar plates in triplicate to enumerate the colony-forming units (CFU). The ratio of CFU to hemacytometer count was usually 0.8.

Pulmonary challenge. Intranasal inoculation was performed as previously described (12). Mice were lightly anesthetized with diethyl ether, and 0.03 ml of yeast suspension was introduced into the nose. Mice were inspected daily for deaths for 8 weeks after challenge. The lungs of dead mice were examined to confirm the cause of death. The characteristic results of fatal infection with *B. dermatitidis* (12), i.e., multiple white nodules and areas of white, caseous consolidation, were confirmed by recovery of large numbers of yeast organisms in culture.

The penetration of the inoculum into the lungs after intranasal challenge was quantitated in three mouse strains. Groups of 10 to 20 mice of each strain were sacrificed by cervical dislocation 10 min after intranasal challenge. Both lungs were removed aseptically and ground in 3 ml of tissue culture medium 199 (GIBCO Laboratories, Grand Island, N.Y.) containing 100 U of penicillin and 100 μg of streptomycin per ml by using glass tissue homogenizers. Samples (1 ml) of serial decimal dilutions of the lung homogenates were placed on sheep blood agar plates in triplicate and incubated at 37°C. Colony counts of yeast grown from the lung homogenates were compared with the count of the inoculum to calculate the percentage of penetration.

s.c. infection. In studies comparing the immune responses of C3H/HeJ and DBA/1J mice, a nonlethal subcutaneous (s.c.) infection was produced by using the parent (virulent) strain of *B. dermatitidis* ATCC 26199. Yeast cells for s.c. inoculation were produced as described for intranasal inoculation, except that the yeast was harvested directly from broth by centrifugation. After three washes in sterile saline, the cells were resuspended in saline to a concentration of 200,000 yeast particles per ml; 0.1 ml of the yeast suspension was injected in two sites over the shoulders to establish s.c. infection.

Blastomyces antigens. Two antigens produced from yeast-phase *B. dermatitidis* were used in the skin test assay, and one was tested in the in vitro lymphocyte proliferation (LP) assay. The blastomyces alkali-soluble, water-soluble antigen was kindly provided by Rebecca Cox (San Antonio State Chest Hospital, San Antonio, Tex.). Its preparation and utility as a skin test antigen in guinea pigs have been previously described (9). A second blastomyces antigen was kindly provided by John E. Bennett (National Institutes of Health). This antigen, referred to as blastomyces lysate antigen or blastomycin, was produced by extracting yeast cells with concentrated urea (11.7 M) in borate buffer (pH 9) for 72 h in the cold. The supernatant was dialyzed against saline, filter sterilized, and adjusted to pH 8 with borate (J. E. Bennett, personal communication). This antigen appeared superior to blastomyces alkali-soluble, water-soluble antigen in LP assays in preliminary studies. Preservative-free *Candida albicans* antigen was obtained from Hollister-Stier Laboratories (Berkeley, Calif.) and used as a negative control antigen.

An additional blastomyces antigen was prepared for use in an enzyme-linked immunosorbent assay for blastomyces antibody. *B. dermatitidis* ATCC 26199 (virulent strain) was grown in the synthetic medium at 37°C on a gyratory shaker (200 rpm) for 7 days. The supernatant was clarified by centrifugation, filter sterilized, and stored at −70°C. Uninoculated medium served as a negative control antigen.

Skin testing. Mice were skin tested by a previously described method (5). Briefly, the mice were prepared by shaving the flank areas with electric animal clippers and applying a foam depilatory (Neet; Whitehall Labs, New York, N.Y.) the day before skin testing. Skin test antigen (0.01 ml) was injected intradermally with a 27-gauge needle and a calibrated Stepper syringe (Indicon, Inc., Brookfield, Conn.). Induration was measured after 24 h with a millimeter rule. Measurements were taken in two directions, and the mean was recorded. Preliminary skin testing experiments were conducted by using several concentrations of each antigen to determine the optimal concentration of each which gave maximal responses in infected mice and minimal responses in normal mice. Based on these findings, the blastomyces alkali-soluble, water-soluble antigen was used in a concentration of 50 μg/0.01 ml, and the blastomyces lysate antigen was used undiluted.

LP of lymph node and spleen cells. Measurement of LP of lymph node and spleen cells in response to several mitogens and fungal antigens was performed

as previously described (6). Briefly, the contralateral inguinal, popliteal, and axillary lymph nodes, as well as spleens, were removed aseptically from three mice of each group. The cells were separated by passing a suspension through fine-meshed screens and were pooled in Eagle minimum essential medium with Earle salts (GIBCO). After settling, the resulting single cell suspensions were washed once with minimum essential medium and resuspended in tissue culture medium, RPMI-1640 with glutamine (GIBCO), containing 100 U of penicillin per ml, 100 μg of streptomycin per ml, 5% (vol/vol) heat-inactivated horse serum (GIBCO), and 5×10^{-5} M 2-mercaptoethanol (Kodak Chemicals, Rochester, N.Y.). Cells were counted in a hemacytometer and adjusted to a concentration of 5×10^6 cells per ml. Triplicate wells of a 96-well round-bottom microtitration tray (Microtest II; Falcon Plastics, Oxnard, Calif.) were filled with 0.1 ml of node or spleen cell pool and either 0.1 ml of medium (control) or 0.1 ml of medium containing mitogen or antigen at twice the final concentration. Trays were incubated at 37°C in an atmosphere of 5% CO_2–95% air. At 20 h before harvest cultures were pulsed with 10 μl of culture medium containing 1 μCi of [*methyl*-^{3}H]thymidine (Schwarz-Mann, Orangeburg, N.Y.; specific activity, 6 Ci/mmol). Cells stimulated with mitogens were cultured for a total of 3 days, and cells with antigens were cultured for 6 days. Cells were harvested on fiber glass filters and counted in a liquid scintillation counter.

Preliminary studies were performed to determine the optimal concentration for each mitogen and antigen. Phytohemagglutinin-P (Difco Laboratories, Detroit, Mich.) was used at a final concentration of 1:800, and concanavalin A (Sigma Chemical Co., St. Louis, Mo.) was used at a final concentration of 5 μg/ml. The optimal concentration of the blastomyces lysate antigen and the candida extract antigen was 1:50 (final dilution).

Antibody testing. Antibody titer to *B. dermatitidis* was determined by using an enzyme-linked immunosorbent assay procedure adapted from previously described methods (4, 29). The blastomyces filtrate antigen was diluted in carbonate coating buffer (pH 9.6) and used to sensitize 96-well polystyrene microtitration plates (Dynatech, Alexandria, Va.). The optimal antigen dilution was determined by block titration (28). After overnight incubation at 4°C, the plates were washed twice with phosphate-buffered saline containing 0.05% Tween 20 (washing solution) and blocked with 4% bovine serum albumin in washing solution at room temperature for 1 h. Serial twofold dilutions of test sera were then prepared in the microtitration plates by using phosphate-buffered saline with 1% Tween 20 (final volume, 200 μl/well) and incubated overnight at 4°C. The test sera were then aspirated, and the plate was washed three times. Horseradish peroxidase-conjugated sheep anti-mouse immunoglobulin G (200 μl; Cappel Laboratories, Cochranville, Pa.) was added to each well and incubated for 30 min at 37°C. The plates were again washed three times with washing solution. Substrate solution containing 0.2 mM 2,2′-azino-di-3-ethyl benzthiazoline sulfonic acid (200 μl; Sigma) and 2 mM hydrogen peroxide in 0.05 M citrate buffer (pH 4.0) was added to each well. The plates were incubated for 30 min at room temperature. The color reaction was stopped with 50 μl of 0.5 M hydrofluoric acid (pH 3.3), and the plates were examined. The final titer was determined as the last well showing a distinct color change.

Statistical analysis. The differences in mortalities observed in the strain survey experiments were tested for significance by using a logistic regression analysis (8). The mean times to death of the various mouse strains were compared by using the Student *t* test. Subsequent experiments comparing a susceptible and a resistant mouse strain were analyzed by using the two-tailed Fisher exact test.

RESULTS

Differences in susceptibilities of inbred strains. In the first strain survey experiment, groups of 12 7-week-old mice of C3H/HeJ, C3H SW/Sn, A/HeJ, C57BL/10J, SJL/J, DBA/1J, and DBA/2J strains were infected intranasally with 8.9×10^4 CFU of attenuated *B. dermatitidis*. The mortalities observed in these seven inbred strains after 56 days of infection ranged from 0 to 83% (Fig. 1). These differences were statistically significant ($P < 0.01$ by logistic regression analysis). Four strains, C3H/HeJ, A/HeJ, DBA/2J, and C3H SW/Sn, were found to be relatively susceptible, with overall mortalities of 83, 75, 75, and 67%, respectively. Deaths in these groups of mice began 4 to 8 days after challenge, and there were few survivors by day 30. SJL/J and C57BL/10J mice were observed to be less susceptible, with mortalities of 33 and 25%, respectively. Deaths in these two strains occurred 2 weeks later in the susceptible strains.

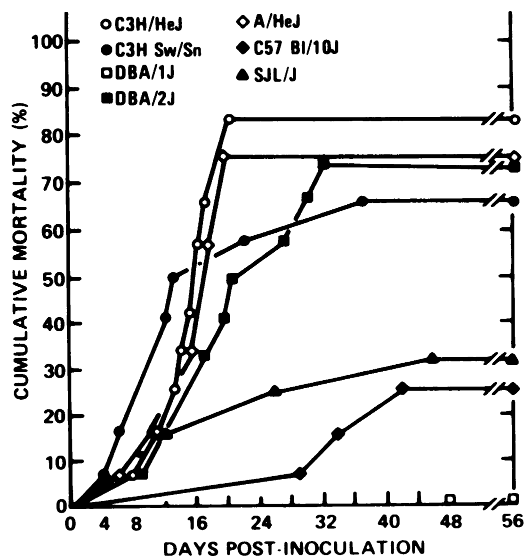

FIG. 1. *Mortality of 7-week-old mice challenged intranasally with 8.9×10^4 CFU of an attenuated strain of B. dermatitidis (12 mice per group).*

The mean time to death of the C3H/HeJ, C3H SW/Sn, A/HeJ, and DBA/2J mice was 16 days, compared with 29 days for SJL/J and C57BL/10J mice ($P < 0.01$ by the Student t test). Finally, no deaths were observed in one strain, DBA/1J, which appeared to be the most resistant of the strains surveyed.

In another experiment with a larger inoculum of 2.5×10^5 CFU to challenge the same seven inbred strains, similar differences in susceptibility were observed (data not shown). C3H/HeJ mice were again found to be the most susceptible, with an overall mortality of 88%. DBA/1J mice, although still the most resistant of the seven strains tested, had a mortality of 38% with the larger challenge, indicating that the resistance of the DBA/1J strain to lethality observed in the first experiment was relative and could be overcome by increasing the size of the inoculum.

To confirm the observation of the initial strain survey that C3H/HeJ mice were more susceptible than DBA/1J mice, groups of 19 to 21 mice of these two selected strains were challenged intranasally with 4.5×10^4 and 1.8×10^5 CFU of attenuated *B. dermatitidis*. In addition, the strain originally used to develop the mouse model of pulmonary blastomycosis (12), BALB/c/St, was also tested. Challenge with 4.5×10^4 CFU was lethal to 80% of the C3H/HeJ mice, compared with 11% of the DBA/1J mice (Fig. 2). This difference was highly significant ($P = 0.0003$ by the Fisher exact test) and clearly establishes that C3H/HeJ mice are more susceptible than DBA/1J mice. The BALB/c/St mice had a mortality of 43% and were of intermediate susceptibility, similar to that observed for SJL/J and C57BL/10J strains in the earlier experiments.

Challenge with larger numbers of yeasts, i.e., 1.8×10^5 CFU, resulted in earlier deaths and higher mortalities in all three strains (data not shown). However, C3H/HeJ mice remained the most susceptible with 82% mortality (by day 18) compared with BALB/c/St (50%) and DBA/1J (21%) mortality at 8 weeks.

Histopathological examination of hematoxylin- and eosin-stained lung tissue from fatal cases from these strains, as well as gross examination, did not suggest differences in the pathological process.

Effect of age on susceptibility. Previous study of murine pulmonary blastomycosis found that younger BALB/c/St mice were more susceptible than older mice of the same strain (12). The susceptibility differences observed between 7-week-old C3H/HeJ and DBA/1J mice might therefore have been due to a difference in relative immunological maturity at the age tested.

To test the effect of age on strain-dependent differences in susceptibility, groups of 4- and 10-week-old (ages relevant to the prior observations cited in BALB/c mice) C3H/HeJ and DBA/1J

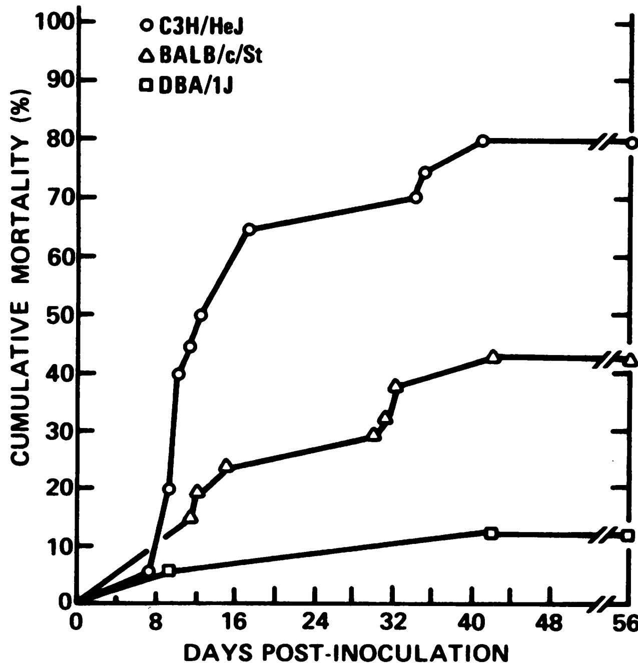

FIG. 2. *Mortality of 8-week-old mice of selected strains challenged intranasally with 4.5×10^4 CFU of an attenuated strain of B. dermatitidis (19 to 21 mice per group).*

mice were challenged simultaneously with 2.2 × 10^4 CFU of attenuated *B. dermatitidis* (Fig. 3). Four-week-old C3H/HeJ mice were considerably more susceptible (83% mortality) than were 10-week-old mice of the same strain (42% mortality). Likewise, 4-week-old DBA/1J mice were relatively more susceptible (25% mortality) than 10-week-old DBA/1J mice (no mortality). However, 10-week-old C3H/HeJ mice were still more susceptible than either 4-week-old or 10-week-old DBA/1J mice. Therefore, these results indicate that the differences in susceptibility of C3H/HeJ and DBA/1J mice observed earlier, at 7 weeks of age, were due to host factors other than different rates of immunological maturation. In all subsequent experiments, 10- to 12-week-old mice were used.

Penetration of yeast into lungs. We tested the possibility that susceptibility differences in mouse strains to intranasal challenge with *B. dermatitidis* might be related to strain-dependent anatomical differences which resulted in varied amounts of the inoculum delivered to the lungs. Ten adult (10- to 12-week-old) C3H/HeJ and DBA/1J mice and 20 adult BALB/c/St mice were given 2.2 × 10^4 CFU intranasally and sacrificed, and their lungs were cultured. The mean recovery (± standard error of the mean) of the inoculum was 30.4 ± 27.1% from C3H/HeJ mice, 41.4 ± 31.4% from DBA/1J mice, and 33.5 ± 27.6% from BALB/c/St mice. These results indicate similar delivery of the inoculum to the lungs of all three mouse strains and do not explain the relative susceptibility of C3H/HeJ mice compared with DBA/1J and BALB/c/St mice.

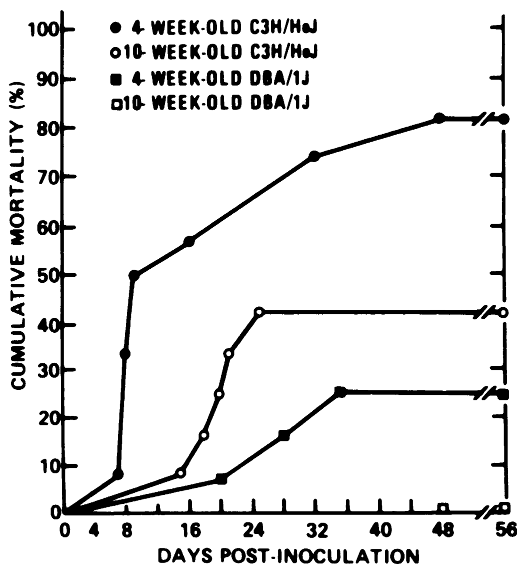

FIG. 3. *Mortality of 4- and 10-week-old mice challenged intranasally with 2.2 × 10^4 CFU of an attenuated strain of B. dermatitidis (12 mice per group).*

Differences in susceptibility of C3H/HeJ and DBA/1J mice to virulent *B. dermatitidis*. Marked differences in virulence in mice have been reported for several strains of *B. dermatitidis* (Brass et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, F33, p. 325). To determine whether the differences in susceptibility of C3H/HeJ and DBA/1J mice were independent of the strain of *B. dermatitidis* used, groups of 16 adult C3H/HeJ and DBA/1J mice were challenged with the parent strain (ATCC 26199) of the attenuated yeast used in earlier experiments. Previous study has shown this strain of *B. dermatitidis* to be highly virulent when given intranasally (12). Initial experiments using challenges of 830 to 17,000 CFU resulted in almost complete mortality in both C3H/HeJ and DBA/1J mice, although the mean time to death of the C3H/HeJ mice was consistently shorter than that of the DBA/1J mice (data not shown). However, at an appropriately smaller inoculum, significant differences in susceptibility were again observed. Intranasal challenge with 80 CFU of virulent *B. dermatitidis* resulted in 88% mortality in C3H/HeJ mice, compared with 38% in DBA/1J mice (Fig. 4). These differences in mortalities of C3H/HeJ mice were significant (P = 0.009) by the two-tailed Fisher exact test.

Susceptibility of C3H/HeJ and C3H/HeN mice. C3H/HeJ mice are reported to possess a defect in activation of macrophages for tumor cytotoxicity and are unresponsive to lipopolysaccharide as well (16). A genetically close subline, C3H/HeN, is histocompatible and does not have these defects (24). Groups of 12 10-week-old C3H/HeJ, C3H/HeN, and DBA/1J mice were challenged with 9.8 × 10^4 CFU of attenuated *B. dermatitidis*. C3H/HeN mice were found to be less susceptible than C3H/HeJ to this challenge (Fig. 5). By day 56, 50% of the C3H/HeJ mice had died of infection, whereas only 25% of the C3H/HeN mice had died. Again, the DBA/1J mice were the most resistant with a lower overall mortality (17%) and a longer mean time to death compared with the C3H/HeJ mice. The DBA/1J mice did not appear significantly different in resistance compared with C3H/HeN mice.

Immune responses of C3H/HeJ and DBA/1J mice to nonlethal infection. The immune responses of C3H/HeJ and DBA/1J mice were studied in an effort to find differences which might be associated with their susceptibility to infection. Initial studies of intranasally infected mice were difficult to interpret due to the lethal nature of the infection. A nonlethal

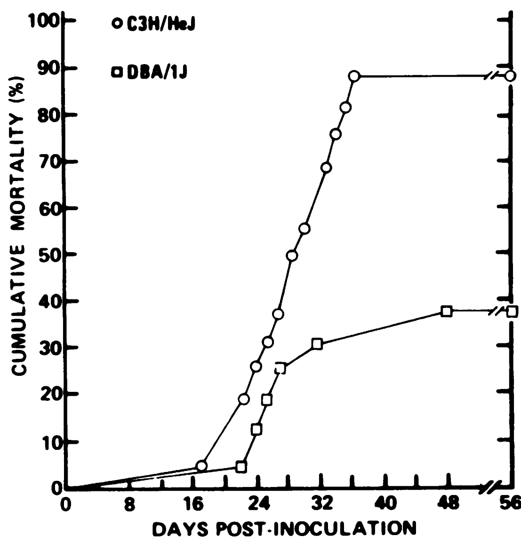

FIG. 4. *Lethality in 10-week-old mice challenged intranasally (80 CFU) with the virulent parent strain of B. dermatitidis.*

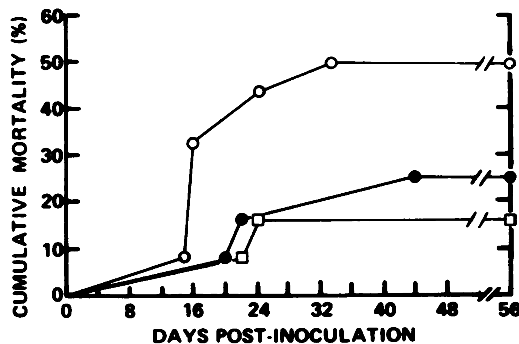

FIG. 5. *Mortality of strains of mice, 10 weeks old, to an attenuated strain of B. dermatitidis (9.8 × 10^4 CFU intranasal challenge). Symbols: □, DBA/1J strain; ○, C3H/HeJ strain; ●, C3H/HeN strain.*

s.c. infection was therefore developed to compare the two mouse strains for the development of delayed-type hypersensitivity, antigen-induced LP, and antibody production. Quantitative culture of s.c. abscesses showed a 3- to 10-fold increase in the number of yeast CFU during the first 1 to 2 weeks of infection and a subsequent marked decline so that viable yeasts were not longer culturable after 4 to 6 weeks (P. A. Morozumi et al., submitted for publication). The peak recovery of organisms and time of clearance of the infection were greater in DBA/1J than in C3H/HeJ mice.

The development of delayed-type hypersensitivity was followed in C3H/HeJ and DBA/1J mice by skin testing with two *B. dermatitidis* antigens at weekly intervals after s.c. infection (Fig. 6). Delayed-type hypersensitivity to both the extracted cell wall antigen, blastomyces alkali-soluble, water-soluble antigen, and the cell lysate antigen (blastomycin) began 1 to 2 weeks after infection and peaked at 3 to 4 weeks. No significant differences were observed between the delayed-type hypersensitivity responses of C3H/HeJ and DBA/1J mice.

Initial attempts to assay LP in lymph node cells early in the course of s.c. infection resulted in very high control counts, possibly due to stimulation by antigen in the draining lymph nodes. Therefore, the LP of lymph node cells and spleen cells of C3H/HeJ mice was compared with that of DBA/1J mice 6 weeks after s.c. infection with two mitogens and two fungal antigens. No significant differences in LP responses of C3H/HeJ and DBA/1J mice to either mitogens or antigens were noted (Table 2). The results show a specific stimulation of lymph node cells and spleen cells by the *B. dermatitidis* lysate antigen but not by the candida (negative control) antigen. Responses to phytohemagglutinin and concanavalin A were similar in both normal and infected C3H/HeJ and DBA/1J mice.

Antibody to *B. dermatitidis* was measured by enzyme-linked immunosorbent assay (Fig. 7). Antibody was detected in C3H/HeJ mice 1 week after s.c. infection and peaked at 4 weeks. In contrast, antibody was not present in DBA/1J mice until 2 weeks after infection and at 4 weeks reached a maximum titer 100-fold less than that of the C3H/HeJ mice.

DISCUSSION

We demonstrated here that mouse strains with different genetic backgrounds differed markedly in their susceptibility to pulmonary blastomycosis. Immunogenetic studies of inbred strains have indicated that the major histocompatibility complex of the mouse, termed *H-2*, is the genetic locus determining ability to make antibody to certain defined antigens as well as some cell-mediated immune reactions (11, 30). The strain survey initially suggested that the *H-2* locus might not be the critical factor in susceptibility to blastomycosis, in that C3H/HeJ and C3H SW/Sn both are susceptible. These two strains differ at the *H-2* locus (Table 1), though both have a C3H genetic background. Strong evidence against *H-2* linkage and against the C3H background as the determinant of susceptibility came from the experiments with C3H/HeJ and C3H/HeN mice. These animals are closely linked in that they are histocompatible, have the same *H-2* locus, can exchange skin grafts, and lack mixed lymphocyte reactivity

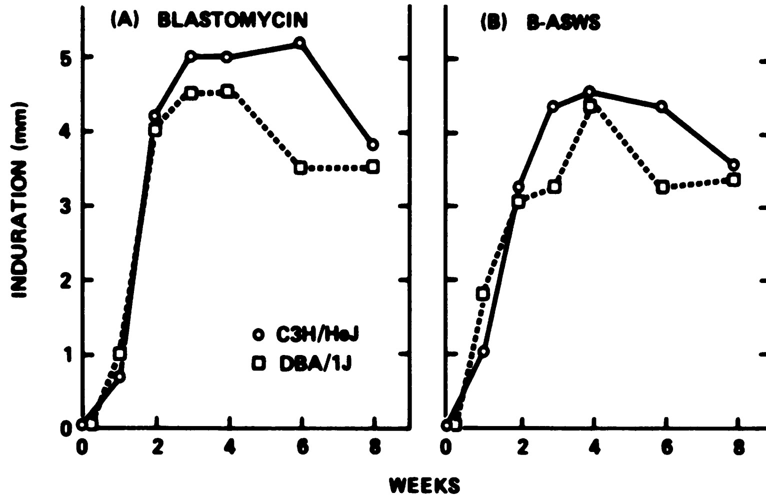

FIG. 6. *Skin test results with two blastomyces antigens (see text) in two mouse strains at various times after the initiation of s.c. blastomycotic infection.*

TABLE 2. *LP of lymph node cells and spleen cells of C3H/HeJ and DBA/1J mice infected s.c. with B. dermatitidis*[a]

Antigen or mitogen	LP in C3H/HeJ mice				LP in DBA/1J mice			
	Normal		Infected		Normal		Infected	
	cpm	Δcpm	cpm	Δcpm	cpm	Δcpm	cpm	Δcpm
Lymph node cells								
Antigen								
None	242 (56)[b]	0	197 (14)	0	192 (39)	0	373 (84)	0
Candida	165 (24)	<1	227 (69)	30	151 (46)	<1	301 (46)	<1
Blasto	227 (77)	35	5,496 (554)	5,299	242 (91)	50	5,864 (1,024)	5,491
Mitogen								
None	216 (63)	0	253 (32)	0	221 (101)	0	346 (95)	0
PHA	4,239 (181)	4,023	5,244 (881)	4,991	2,112 (147)	1,891	3,456 (500)	3,110
ConA	3,625 (51)	3,409	4,839 (520)	4,586	2,017 (479)	1,796	7,405 (895)	7,059
Spleen cells								
Antigen								
None	135 (42)	0	294 (55)	0	139 (39)	0	256 (78)	0
Candida	154 (39)	19	218 (44)	<1	148 (24)	9	205 (35)	<1
Blasto	178 (6)	43	1,297 (236)	1,004	147 (22)	8	1,775 (110)	1,519
Mitogen								
None	176 (62)	0	185 (45)	0	133 (13)	0	196 (24)	0
PHA	3,284 (200)	3,108	3,587 (798)	3,402	744 (70)	611	964 (17)	768
ConA	4,302 (505)	4,126	6,152 (772)	5,967	4,155 (668)	4,022	2,308 (212)	2,096

[a] Abbreviations: cpm, counts per minute; Δcpm, cpm with antigen or mitogen minus cpm in absence of these; PHA, phytohemagglutinin; ConA, concanavalin A; blasto, blastomyces lysate antigen. See text for sources and concentrations of reagents used.

[b] Numbers within parentheses indicate the standard error.

(24); yet their susceptibility was markedly different. The lack of correlation of susceptibility with *H-2* is compatible with data with other infectious agents, e.g., measles (17), listeriosis (7, 27), and salmonellosis (18). Further studies with congenic strains in this model would confirm our observations.

The differences in susceptibility we demon-

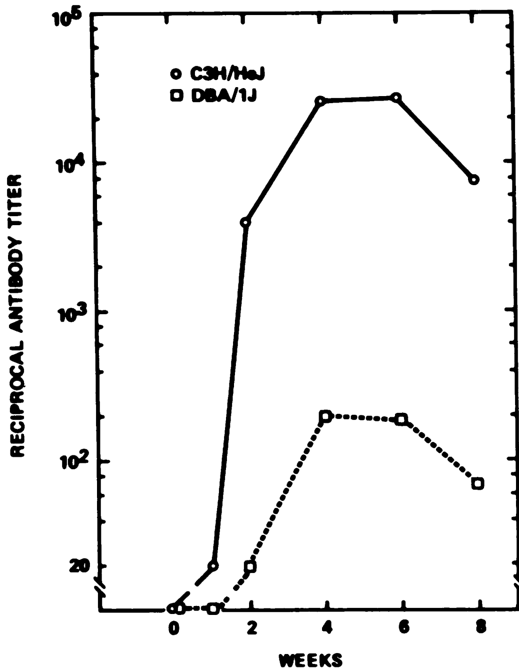

FIG. 7. *Titers of antibody to blastomyces antigen determined by the enzyme-linked immunosorbent assay (see the text) at various times after the initiation of s.c. blastomycotic infection.*

strated were shown (i) with several challenge sizes, (ii) with different blastomyces strains, and (iii) at different levels of host maturation. The differences are also not due to mechanical factors, such as the number of CFU reaching the lower respiratory tree.

In studying the possibility that immunological differences between strains would be responsible for susceptibility differences, we felt it important to use comparable challenge doses, which, by the pulmonary route, would have undesirably produced infections of varied severity in different strains. It was also deemed important to avoid the pitfall of studying immune functions in the face of infectious processes that are asymmetric between strains (21). In that situation, even if the infection in both strains is self-limited, enhanced replication of the pathogen in the susceptible strain could stimulate the immune parameters being measured. One might infer erroneously from this that immune reactivity is greater in the susceptible strain. Alternatively, in a disease process which progressed in the susceptible strain, the debility accompanying infection could suppress immune functions being measured. This could lead to a conclusion that the depressed immune function was responsible for the progression. For this reason, a nonprogressive infection (s.c.) was studied and shown comparable between the strains, and the immune functions were assayed in this setting. No differences in cell-mediated immunity as assayed by delayed-type hypersensitivity or LP with either lymph node or spleen cells and with two different antigens were demonstrated. Antibody production was delayed in the resistant strain and reached a much lower titer. It is possible that some of the increased antibody might block or inhibit an important defensive effector cell, and this is worthy of further study.

The studies with C3H/HeJ and C3H/HeN mice may give a clue as to the differences in susceptibility demonstrated. The C3H/HeJ strain is known to lack a response to several biological functions mediated by lipopolysaccharide, due to a mutation occurring in an inbred colony in the 1960's (25). These animals also have a macrophage defect, as expressed in cytotoxicity (25), and cross-breeding experiments have suggested that this and the lipopolysaccharide defect are mediated by the same gene (26). The C3H/HeJ strain, however, is not uniformly more susceptible to infectious diseases. For example, mice of this strain are susceptible to listeriosis (7), salmonellosis (18), and rickettsial infection (16), but resistant to herpes simplex (14) and viral encephalomyelitis (15) infections. The susceptibility to listeriosis is not apparently linked to the macrophage defect, since other mice with C3H background lacking the defect are also susceptible (7), unlike our results; however, cross-breeding experiments have suggested that the susceptibility to salmonellosis is mediated by the same gene as that mediating the lipopolysaccharide defect (18) and, thus, is probably linked to the macrophage defect (26). It should also be noted that with the present study, differences in susceptibility to infection between these closely related C3H strains have now been shown for fungal as well as viral (14) and bacterial (18) infections.

Further evidence for a possible role for macrophages in the susceptibility differences studied here is suggested by the result in the strain survey showing the A/HeJ strain to be the second most susceptible strain. Mice of A background also have a macrophage defect (1), although there are differences from the defect present in C3H/HeJ mice, and these defects appear to be mediated by different genes (2, 16). However, the situation is not simple, since studies with tuberculosis (22) and leishmaniasis (3) have suggested that A strain mice are resistant (whereas DBA/1 mice are susceptible). Studies of a possible macrophage difference between the strains are in progress. Other data needed include whether the susceptibility of C3H/HeJ

mice is sex linked (the macrophage and lipopolysaccharide defects are not), as the present studies have been completed with male mice.

A recent study of the genetics of resistance in mice to *Cryptococcus neoformans* (23) indicated that susceptibility is related to the absence of complement component C5. This does not appear to be the explanation for susceptibility to blastomycosis, since our most susceptible and resistant strains, C3H/HeJ and DBA/1J, respectively, were both resistant to cryptococcosis and have C5, and other strains we surveyed and found intermediate in resistance to blastomycosis are susceptible to cryptococcosis and lack C5.

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