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# NOTES

## Strain Differences in Resistance to Infection Reversed by Route of Challenge: Studies in Blastomycosis

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The inbred mouse strains C3H/HeJ and DBA/1J have been shown to represent the extremes of susceptibility and resistance, respectively, to pulmonary blastomycosis. This pattern was completely reversed when challenge was performed by the intraperitoneal route, whether a virulent or an attenuated strain of *Blastomyces dermatitidis* was utilized. By a third route (subcutaneous), the differences were insignificant. Inhibition of replication of blastomyces in vitro by macrophages from both strains, before or after activation by subcutaneous infection, was similar.

We have previously reported in studies with a murine model of pulmonary blastomycosis (2) that, of nine strains of mice studied, the C3H/HeJ strain was the most susceptible and the DBA/1J strain was the most resistant (3). The significant differences between the C3H/HeJ and DBA/1J strains occurred at several challenge sizes, were not dependent on the strain of *Blastomyces dermatitidis*, host age, or ability of the challenge inoculum to penetrate to the lower airways, and did not appear to be related to their differences in the *H-2* locus or to deficiencies in complement. For example, intranasal challenge (2) of 10-week-old mice with 80 colony-forming units (CFU) of the virulent strain *B. dermatitidis* ATCC 26199 was shown to result in 88% mortality in 8 weeks in C3H/HeJ mice, as opposed to 37% of DBA/1J mice ($P = 0.009$). This was confirmed with a mutant (3), attenuated in virulence, of this strain of *Blastomyces*. We wanted to determine whether these differences in susceptibility were dependent on the route of challenge.

In these and the experiments to be described, the two-tailed Fisher exact method was used to analyze statistical significance. Groups of 12 10-week-old male mice were challenged concurrently in each series of experiments and observed for 8 weeks. The groups of mice were obtained from the same supplier (Jackson Laboratories, Bar Harbor, Maine) at the same age, housed in the same room in our facilities, and received the same diet (3). Postmortem examinations and cultures of the lungs and peritoneal cavities were performed to confirm the presence of characteristic blastomycotic nodules (2).

Yeast cells for inoculation were prepared by seeding cells from refrigerated slants to a liquid synthetic medium (2), which was then incubated at 37°C on a gyratory shaker (200 rpm) for 72 to 96 h. The cells were passaged once in the medium to achieve log-phase growth (72 h), then transferred to sheep blood agar plates, and incubated at 37°C for 72 h. The cells were harvested, washed in saline, and counted in a hemacytometer. Dilutions were placed on blood agar plates in triplicate to enumerate the CFU in the challenge. The ratio of CFU to hemacytometer count by these methods is approximately 0.8.

In marked contrast to pulmonary challenge, intraperitoneal infection (accomplished with 0.3-ml volumes) with different *B. dermatitidis* strains produced entirely different results. Mice were challenged intraperitoneally with 123,000 CFU of the attenuated mutant strain (Fig. 1A), which produced only 8% deaths in 8 weeks in C3H/HeJ mice, as opposed to 75% in DBA/1J mice ($P = 0.0027$), a complete reversal of the susceptibilities of these mice to intranasal infection. A subsequent experiment with an intraperitoneal challenge of 190 CFU of the virulent strain, ATCC 26199, resulted in 25% lethality in C3H/HeJ mice and 100% deaths in DBA/1J mice ($P = 0.0003$; Fig. 1B).

Because the above experiments were performed at various times, a confirmatory experiment was performed with two groups of C3H/

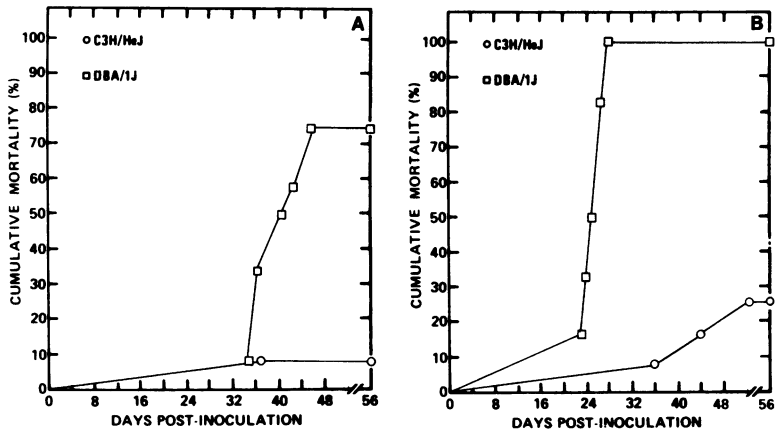


FIG. 1. *Groups of mice challenged intraperitoneally with B. dermatitidis and observed. (A) Challenge of 123,000 CFU attenuated strain; (B) 190 CFU, virulent strain. Symbols: O, C3H/HeJ strain; □, DBA/1J strain.*

HeJ and DBA/1J mice which were challenged either intranasally or intraperitoneally with the same inoculum, 30 CFU, of virulent *B. dermatitidis*. C3H/HeJ mice were again more susceptible (50% mortality) than DBA/1J mice (25%) to intranasal challenge; however, the C3H/HeJ mice were less susceptible (17%) to the same challenge when it was given intraperitoneally compared with intranasal challenge. In sharp contrast, the DBA/1J mice were again found to be highly susceptible (83% mortality) to intraperitoneal infection ($P = 0.0037$). As expected, the mortality in both strains by both routes in this experiment was slightly less than that in the experiments described above, in which slightly larger challenges were used.

A third route of infection, subcutaneous challenge, was therefore investigated. Subcutaneous infection with the virulent strain of *B. dermatitidis* was nonlethal and spontaneously resolved in both strains. Groups of mice were then challenged subcutaneously with 19,000 CFU of the virulent strain, *B. dermatitidis* ATCC 26199. The course of the infection was followed by quantitatively culturing abscesses removed from mice at various times after challenge. In this experiment, groups of 15 mice were used. Three mice were sacrificed weekly, and the resultant abscess in each was excised in toto, minced, ground in a glass tissue grinder, and agitated in a Vortex mixer to produce a fine suspension (2). The suspension from each mouse was assayed for CFU with decimal dilutions in triplicate on blood agar plates (Fig. 2). A higher mean recovery (at 2 weeks after injection) was observed in DBA/1J mice than in C3H/HeJ mice. Recovery from C3H/HeJ mice peaked at 1 week of infection and also declined in C3H/HeJ mice at a faster rate. By 4 weeks, both strains completely

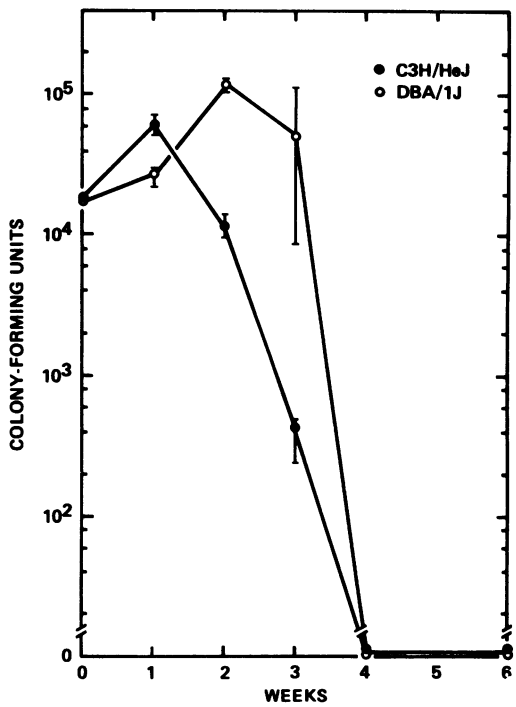


FIG. 2. *Groups of mice challenged subcutaneously with the virulent B. dermatitidis strain. Mice were sacrificed weekly, and the resultant abscesses were cultured quantitatively for fungus. Data presented with standard error of the mean of three abscesses.*

cleared the subcutaneous infection, and no viable yeast cells were found at either 4 or 6 weeks. Similar results were obtained from a second experiment with a slightly larger challenge (data not shown).

In an attempt to elucidate the mechanism of the susceptibility differences demonstrated, we

examined the ability of peritoneal macrophages from both strains to inhibit the replication of *B. dermatitidis* in vitro. We have previously shown (1) in BALB/c mice (a strain intermediate in resistance to pulmonary challenge) that these cells can inhibit the growth of the fungus in vitro in 24 h cocultures and that subcutaneous infection, as performed in the experiments just described, markedly enhanced this property. The growth inhibition was shown not to be due to depletion of constituents of the medium by the macrophages. Because of the strain differences in resistance to intraperitoneal challenge described, we studied this function in peritoneal macrophages taken from both C3H/HeJ and DBA/1J mice before and serially after subcutaneous infection. Macrophages were elicited by lavage with Eagle minimal essential medium and selected by 3-h adherence to plastic. The CFU per well after 24 h of coculture were compared with growth without macrophages, measured by enumeration of CFU on blood agar plates (1). Inhibiting power increased in the first weeks after infection and remained high for up to 6 to 8 weeks, but there were no consistent significant differences between the two strains (Table 1). Similar data were obtained when the attenuated strain was used as the macrophage target in vitro. The macrophage inhibitory data described and the changes at intervals after subcutaneous infection are also not different from what we have determined with BALB/c cells (data not shown).

Strain differences in mice in susceptibility to microbial pathogens have been demonstrated with a variety of viruses, bacteria, protozoa, and fungi, and in some instances, the gene loci determining this have been defined. However, to our knowledge, with one exception (4), these differences have either not been tested or not been demonstrated with regard to differences in results varying with the route of challenge. Turcotte (4) showed some evidence of reversal of mouse strain susceptibility after challenge with *Mycobacterium lepraemirum* by different routes, but the reversal was not as profound as that demonstrated here. Our data are striking in that significant differences in resistance between strains to challenge by one route were accompanied not only by a loss of these differences but also by inverse and significant differences when challenge was performed by a second route. By a third (subcutaneous) route of challenge, the differences between the strains were minimal. These results suggest genetically determined resistance factors which are different in different anatomical loci. The factors responsible for the differences in resistance demonstrated have not been defined at this time.

TABLE 1. *Inhibition of fungal replication by macrophages from two mouse strains*

| Wk | Inhibition of *B. dermatitidis*[a] plus: | | |
|---|---|---|---|
| | Medium | Macrophages[b] | |
| | | C3H/HeJ | DBA/1J |
| 0[c] | 2,267 ± 450[d] | 2,333 ± 238 (0)[e] | 2,180 ± 423 (4) |
| 1 | 5,127 ± 670 | 2,753 ± 704 (46) | 3,273 ± 353 (36) |
| 2 | 6,533 ± 686 | 2,440 ± 454 (63) | 1,753 ± 283 (73) |
| 3 | 3,200 ± 340 | 1,493 ± 150 (53) | 713 ± 133 (78) |
| 4 | 6,140 ± 506 | 1,893 ± 102 (69) | 1,633 ± 75 (73) |
| 6 | 6,373 ± 699 | 2,907 ± 53 (54) | 2,127 ± 549 (67) |
| 8 | 7,020 ± 711 | 3,860 ± 245 (45) | 2,020 ± 556 (71) |

[a] Mean inoculum ± standard deviation at 0 h in seven experiments, 451 ± 130 CFU.
[b] A total of 500,000 adherent peritoneal cells per well, in triplicate, from three mice of each strain each week.
[c] Weeks after subcutaneous infection with *B. dermatitidis*.
[d] CFU per culture ± standard deviation on blood agar after 24 h of coculture of *B. dermatitidis* and macrophages.
[e] Percent inhibition of replication.

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