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Strain-Dependent Differences in Host Response to *Candida albicans* Infection in Mice Are Related to Organ Susceptibility and Infectious Load

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After systemic infection with the yeast *Candida albicans*, inbred mice show substantial differences in mortality, organ colonization, and severity of tissue damage. To examine the relationships between these variables, which are not directly correlated with each other, fungal colonization of the kidneys and brain was enumerated in six inbred strains that exhibit different patterns of tissue damage and mortality. Mice lacking the fifth component of complement (C5) are highly susceptible to lethal challenge, and A/J and DBA/2 mice, both C5 deficient, showed the highest colony counts in the kidneys after challenge with 10^5 blastoconidia. In contrast, colony counts in the brains of all six strains were equivalent at this challenge dose. A/J and DBA/2 mice died after challenge with 3×10^5 blastoconidia, but other strains showed an increase in kidney colonization, and strain-dependent differences in clearance from the brain became evident. The data suggest that mortality in A/J and DBA/2 mice is related to an unusual susceptibility of the kidneys to colonization by *C. albicans* and that there may be tissue-specific differences in host protective mechanisms.

Genetically defined inbred mice show substantial differences in mortality and in colony counts in the infected kidneys following intravenous challenge with the yeast *Candida albicans* (4, 6, 7). A deficiency in the fifth component of complement (C5) appears to be the dominant genetic feature that increases susceptibility to a lethal challenge (4). Although there is a loose correlation between mortality and fungal burden as measures of infection (6, 10), histological assessment of the lesions has shown a dichotomy in patterns of tissue destruction in the brains and other organs of different inbred mice (2). A further survey of tissue damage, mortality, and *Candida* colonization in the brains of eight inbred strains did not demonstrate any correlation between the various measures of susceptibility (1). Although C5-deficient mice showed a slight increase in fungal colonization of the brain, the deficiency bore no relationship to the development of a pattern of mild or severe tissue damage. These observations suggested that the different measures of infection reflect different aspects of the host response.

To investigate this question, patterns of fungal colonization of and clearance from the brain and kidneys were compared in the inbred strains A/J (*H-2*^{a}, C5^0), AKR (*H-2*^{k}, C5^0), BALB/c (*H-2*^{d}, C5^1), DBA/1 (*H-2*^{q}, C5^1), DBA/2 (*H-2*^{d}, C5^0), and CBA/CaH (*H-2*^{k}, C5^1). The mice were purchased from the Animal Resources Centre, Perth, Australia, and were bred under specific-pathogen-free conditions. Animals were infected intravenously with *C. albicans* blastoconidia (isolate 3630 from the Mycology Reference Laboratory, Royal North Shore Hospital, Sydney, Australia) in a 0.2-ml volume via the tail vein. Only female mice, 6 to 8 weeks of age, were used. In any particular experiment, three mice of each strain were inoculated with the same preparation of *C. albicans* and each experiment was repeated with a separate preparation. Approval was obtained from the University of Western Australia Animal Experimentation Ethics Committee which required that the minimum number of animals needed to obtain statistical validity be used in any individual experiment.

Mice were sacrificed at various times after infection, and brains and kidneys were removed for histological examination and quantitative culture. A portion of each organ was fixed in formalin, sectioned, and stained with hematoxylin and eosin or periodic acid-Schiff. Random sections were coded, examined blind, and reevaluated when the code had been broken. The remainder was weighed and homogenized in phosphate-buffered saline with an Ultra Turrax T-25 homogenizer (IKA Labortechnik, Staufen, Germany) at 13,500 rpm and room temperature. The samples were diluted appropriately, and 100-μl aliquots were plated in duplicate on Sabouraud agar containing chloramphenicol. The plates were incubated at 37°C for 2 days, and the colonies were counted. The results were expressed as CFU per gram of tissue. The data from at least two experiments were pooled, giving a minimum of six mice for each dose and each time point. Comparisons were made by using the Student *t* test or the Scheffe test on the one-way analysis of variance.

A/J and DBA/2 mice are more susceptible than other strains to lethal systemic infection (1, 4) and died after challenge with 3×10^5 blastoconidia (data not shown). However, all mice survived infection with 10^5 blastoconidia. Histological examination of the kidneys revealed the presence of acute pyelonephritis in infected mice of all six strains. The most severe lesions were seen in C5-deficient DBA/2 (Fig. 1A), A/J, and AKR mice, whereas BALB/c mice were the least affected (data not shown). Yeast cells, mycelia, and fungal debris were most obvious in the kidneys of mice, such as the DBA/2 strain, that developed severe pyelonephritis, whereas in the congenic DBA/1 mice (Fig. 1B) the lesions were smaller and periodic acid-Schiff-positive material was not demonstrated. Polymorphonuclear leukocytes dominated the inflammatory infiltrate in CBA/CaH and BALB/c mice (data not shown) but were less obvious in the renal lesions of A/J, DBA/2, and AKR mice. The infected kidneys in these three strains demonstrated a

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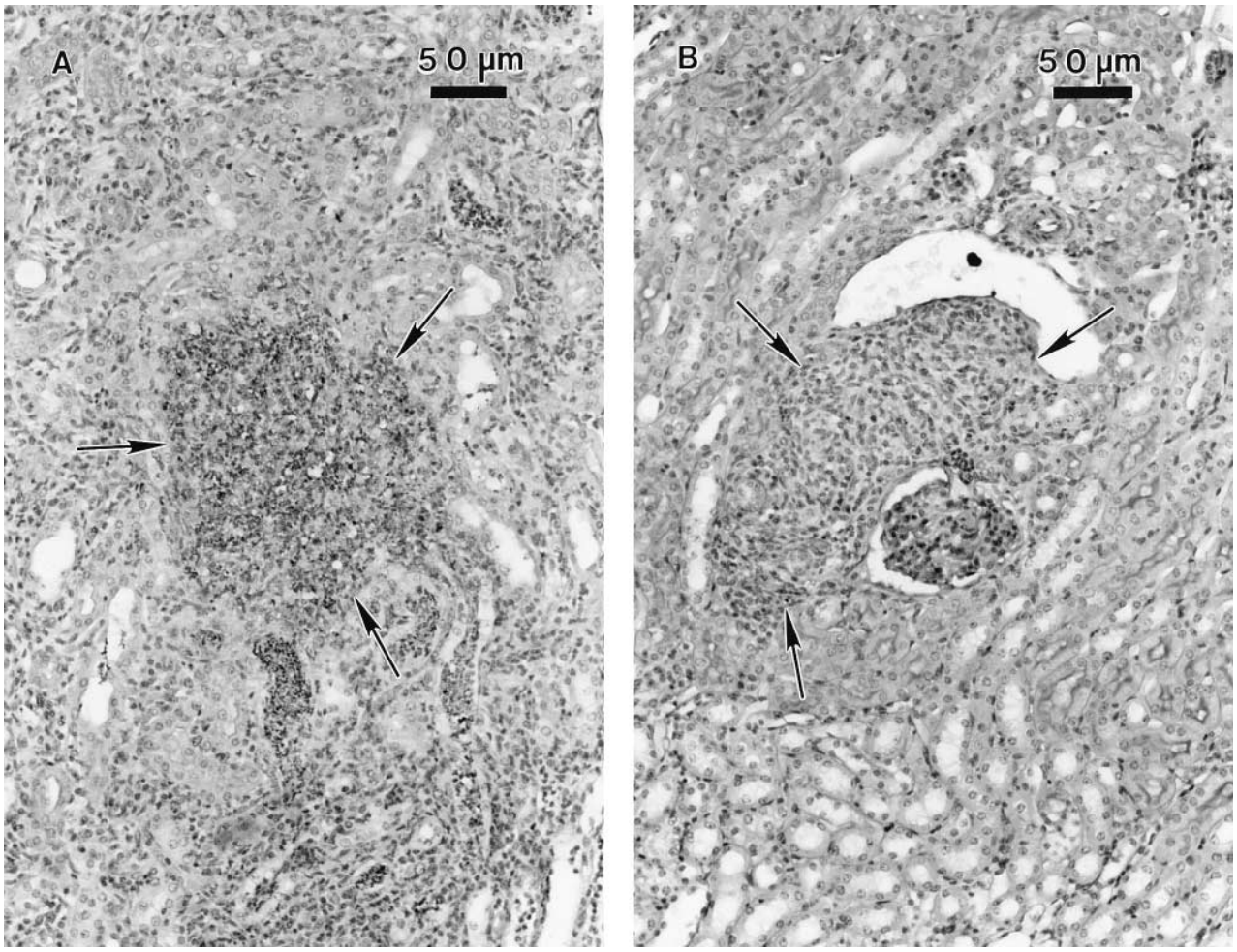

FIG. 1. Histological appearance of lesions (arrows) in the kidneys of DBA/2 (A) and DBA/1 (B) mice 5 days after intravenous infection with 10^5 *C. albicans* blastoconidia. Periodic acid-Schiff stain was used.

substantial granulomatous component, which was also found to a significant degree in the kidneys of infected DBA/1 mice.

Ranking of strains by survival alone (6) agrees with differential susceptibility as assessed by quantitative culture of the kidneys (10), suggesting that the ability of the kidney to limit the growth of the yeast may be the crucial factor in determining mortality after infection. The kidneys possess efficient phagocytic defenses, which are capable of eliminating large amounts of *Candida* cells within the first few hours after infection (3). However, with large inocula, destruction of the yeast cells occurs only within the first 3 h, after which time the organisms multiply, and death ensues when the fungal burden reaches a point at which the kidneys fail (5). To determine whether the increased severity of tissue damage and associated mortality (9) in C5-deficient mice is associated with more rapid onset and development of infection, kinetic studies were carried out with strains A/J ($C5^0$), CBA/CaH, and BALB/c. These last two are C5 sufficient but show patterns of severe and mild tissue damage, respectively (2).

A/J mice showed higher levels of colonization (Fig. 2), but the overall patterns of infection of all three strains were similar. The fungal burdens in the kidneys of the six inbred strains were compared 5 days after infection by using graded challenge doses. After infection with 10^5 blastoconidia, significantly more colonies ($P < 0.01$) were recovered from the kidneys of A/J, DBA/2, and AKR mice than from those of C5-sufficient strains (Fig. 3), but A/J and DBA/2 mice showed higher levels of colonization than AKR mice ($P < 0.05$). When the inoculum was increased to 3×10^5 blastoconidia, colony counts in the four surviving strains increased but, except in AKR mice, did not reach levels comparable to those seen in A/J and DBA/2 mice inoculated with 10^5 or 5×10^4 blastoconidia (Fig. 3).

This suggests that the innate resistance of the kidneys in the AKR strain may be reinforced by immunologically mediated protective mechanisms that increase clearance and improve survival. If the excessive mortality of strains such as DBA/2 and A/J is caused by an unusually large accumulation of *Candida* cells in the kidneys, leading to renal failure, then survival may be increased by augmentation of local phagocytic defense mechanisms, such as by treatment of the mice with muramyl dipeptide (8). However, this decreased mortality may not necessarily be linked to, or reflect, protective events occurring at other sites, so that a challenge that overloads a particularly susceptible organ may preempt and prevent detection of a potentially successful response in other organs or tissues.

Such effective tissue responses were evident when clearance of *Candida* cells from the brains of the six strains was investi-

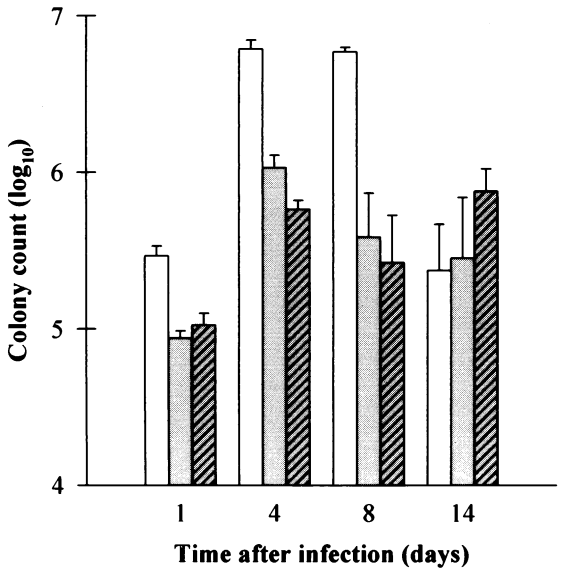

FIG. 2. Clearance of *C. albicans* from the kidneys of inbred mice after intravenous challenge with 10^5 blastoconidia. Each bar represents the mean ± the standard error of duplicate determinations from a minimum of six mice. The fungal burden in A/J mice was significantly greater than that in the other two strains at days 1, 4, and 8 ($P < 0.01$ at each time point as determined by Scheffe's test on the analysis of variance). ▭, A/J; ▭, BALB; ▨, CBA.

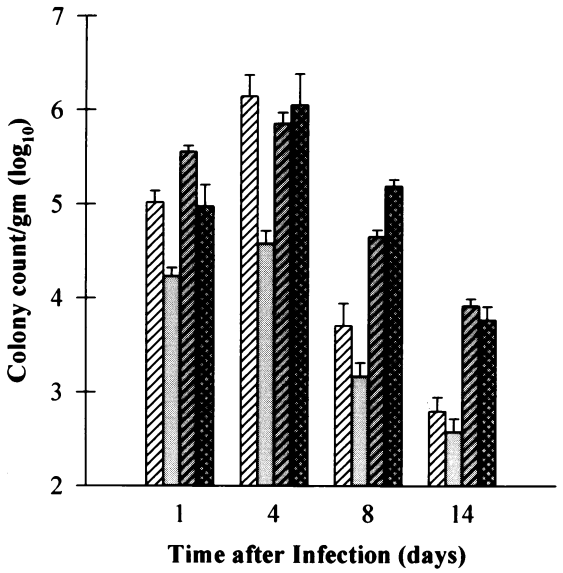

FIG. 4. Clearance of *C. albicans* from the brains of inbred mice after intravenous challenge with 3×10^5 blastoconidia. Each bar represents the mean ± the standard error of duplicate determinations from a minimum of six mice. There were significantly fewer yeast cells in the brains of BALB/c mice than in those of the other strains at days 1 and 4 after infection ($P < 0.05$ by Scheffe's test on the analysis of variance), whereas CBA/CaH and DBA/1 mice maintained a significantly higher ($P < 0.05$) level of colonization than BALB/c and AKR mice at days 8 and 14. ▨, AKR; ▭, BALB/c; ▨, CBA/CaH; ▨, DBA/1.

gated. In contrast to the kidneys, after intravenous infection with 10^5 blastoconidia, growth curves in the brains of all six strains were virtually identical (data not shown). However, when mice were challenged with 3×10^5 blastoconidia (Fig. 4), colony counts reached a peak on day 4 but CBA/CaH and DBA/1 mice showed slower clearance, with significantly higher colony counts, at the later sampling times. Variation in fungal burden in the brains of the different strains as the inoculum of *C. albicans* is increased suggests a hierarchy not only in the innate susceptibility to colonization of different tissues and organs but also in the efficiency of the local host response.

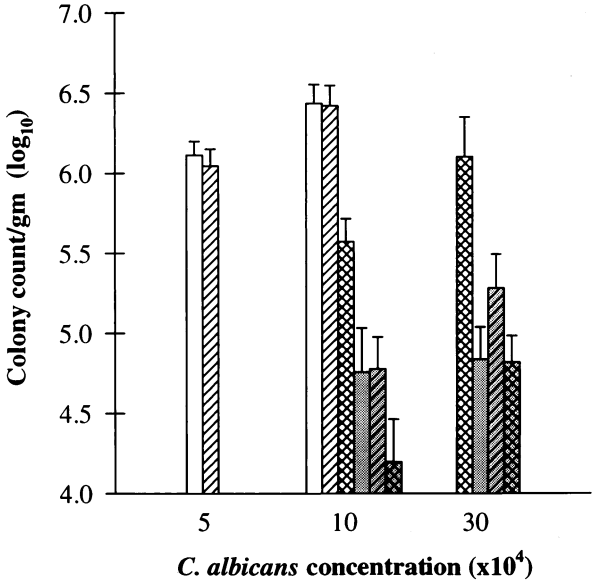

FIG. 3. Fungal burdens in the kidneys of inbred mice 5 days after intravenous infection with either 5×10^4, 1×10^5, or 3×10^5 *C. albicans* blastoconidia. Each bar represents the mean ± the standard error of duplicate determinations from a minimum of six mice. Analysis of variance showed that the groups given 1×10^5 blastoconidia differed significantly ($P < 0.01$) in kidney colonization by the yeast. Overall, C5-deficient mice had higher counts than C5-sufficient strains ($P < 0.01$), and strain A/J and DBA/2 counts were higher than strain AKR counts ($P < 0.05$). Similarly, in the groups given 3×10^5 blastoconidia, colony counts were significantly higher ($P < 0.05$) in C5-deficient AKR mice than in the other three strains. ▭, A/J; ▨, DBA/2; ▨, AKR; ▭, BALB/c; ▨, CBA; ▨, DBA/1.

These quantitative variations in the amounts of *Candida* cells in the tissues occur within the context of genetically determined differences in susceptibility to tissue damage (2) but are not related to it and do not show any obvious correlation with identifiable genetic loci in the strains studied. In view of these data, it is important to consider not only strain variation but also organ susceptibility in the interpretation of experimental models of candidiasis.

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