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Role of Complement During Experimental *Candida* Infection in Mice

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The role of complement during experimental *Candida albicans* infection was investigated in mice. It was found that complement-positive CF-1 mice, infected with the test organism, survived significantly longer than complement-deficient mice of the same strain. This implies the participation of the relatively late acting complement component in host defense mechanisms directed against this systemic mycotic infection.

The role of the complement (C) system in resistance to disease has long been difficult to demonstrate, especially in a complex situation such as the host-pathogen relationship. With the recognition of animal strains deficient in some C component, the task of elucidating its in vivo functions has been made easier. Inbred strains of mice and individuals of non-inbred strains which lack the complete C system were previously described (12, 16). The mouse then has become an animal which readily offers an experimental system to test the importance of certain components of this complex series of serum proteins. The component which is missing in C-deficient mice was shown to be C5 (11). Two reports using deficient and "normal mice" presented evidence that C plays a role in resistance to experimental bacterial infections. The first report showed that C-deficient mice are more susceptible to infection by *Corynebacterium kutscheri* compared to normal mice (1). The second group demonstrated that the complete C system is of survival value for mice infected intravenously with *Diplococcus pneumoniae* (15).

The pathogenesis of fungal infections is relatively poorly understood compared to the understanding of the pathogenetic processes in many bacterial and viral infections. Thus, the reliability of assessment of individual fungal substances as "aggressins" or of particular host defense mechanisms as being of special importance is at present poor. The importance of the complement system in mycotic infections has been assessed little if at all. In this report we present evidence that C plays a role in defense against experimental infection in mice caused by *Candida albicans*.

MATERIALS AND METHODS

Organisms. The organism used throughout this investigation was *C. albicans* strain FF-100 obtained from the departmental stock collection.

Animals. The animals used for the in vivo experiments were CF-1 female mice, 8 to 10 weeks of age, obtained from Carworth Farms, Inc.

Test for complement. The serum of each mouse was tested for hemolytic complement by using the system of Rosenberg and Tachibana (13). There is no ambiguity in test results, and mice were assigned to experimental groups designated as complement positive (C^+) and complement deficient (C^-).

Method of infection. Groups of C^+ and C^- mice were injected intravenously (iv) or intraperitoneally (ip) with nonpyrogenic physiological saline containing increasing numbers of *Candida* cells grown for 24 hr at 37 C in fluid Sabouraud's medium. Yeast counts were made by hemocytometer and checked by pour plates with Sabouraud's glucose agar. The animals were observed every day for 28 days, and LD_{50} values were calculated.

RESULTS

LD_{50} determinations. Groups of eight mice (of each type) were injected iv with suspensions of *Candida* containing from 1.4×10^4 to 2.8×10^6 cells. Table 1 shows accumulated mortality at different dose levels. LD_{50} values [determined by the Spearman-Kärber estimator method (2)] were 1.8×10^5 for C^+ mice and 1.0×10^5 for C^- mice. Animals injected ip survived up to 1.6×10^6 *Candida* whether they were C^+ or C^-.

Survival of C^+ and C^- mice. Although the LD_{50} values were not significantly different, with moderate to high challenging doses, the C^+ mice survived longer than C^- mice. Results obtained at six different doses [analyzed by the Wilcoxon test (17)] indicate that the survival rate of C^- mice is significantly lower than that of C^+ mice. The results are given in Fig. 1. The left side of the figure gives the results obtained when groups of eight mice were challenged with numbers of organisms indicated therein. The right half of the figure gives the results of a second experiment in which groups of 15 mice were injected. There is complete concordance between the two experi-

TABLE 1. *Mortality of C+ and C− CF-1 female mice injected intravenously with various doses of Candida albicans*[a]

Injected dose	Death of mice at 28 days	
	C+	C−
2.8×10^6	8/8	8/8
1.4×10^6	8/8	8/8
7.0×10^5	6/8	7/8
2.8×10^5	4/8	7/8
1.4×10^5	4/8	4/8
5.4×10^4	2/8	3/8
2.8×10^4	1/8	3/8
1.4×10^4	0/8	0/8

[a] Groups of eight mice were challenged with the numbers of organisms indicated. They were observed for 28 days thereafter. The LD_{50} for C+ mice is 1.8×10^5 and for C− mice is 1.0×10^5.

ments. The *P* values at each dose level are highly significant. They are shown in the figure.

We carried out many experiments attempting to ascertain the mechanism of loss of resistance in C− mice. Since these efforts have thus been unavailing, we present the results and our conclusions in general terms in the discussion.

DISCUSSION

The results obtained by iv inoculation of *C. albicans* into mice demonstrate that C plays an important role in resistance to infection. This resistance is seen optimally at dose levels high enough to kill most of the inoculated mice. At lower doses most of the mice in both groups survive, and the difference is less apparent.

The precise mechanism of C function in resistance to experimental infection is unknown. Results obtained from in vitro experiments failed

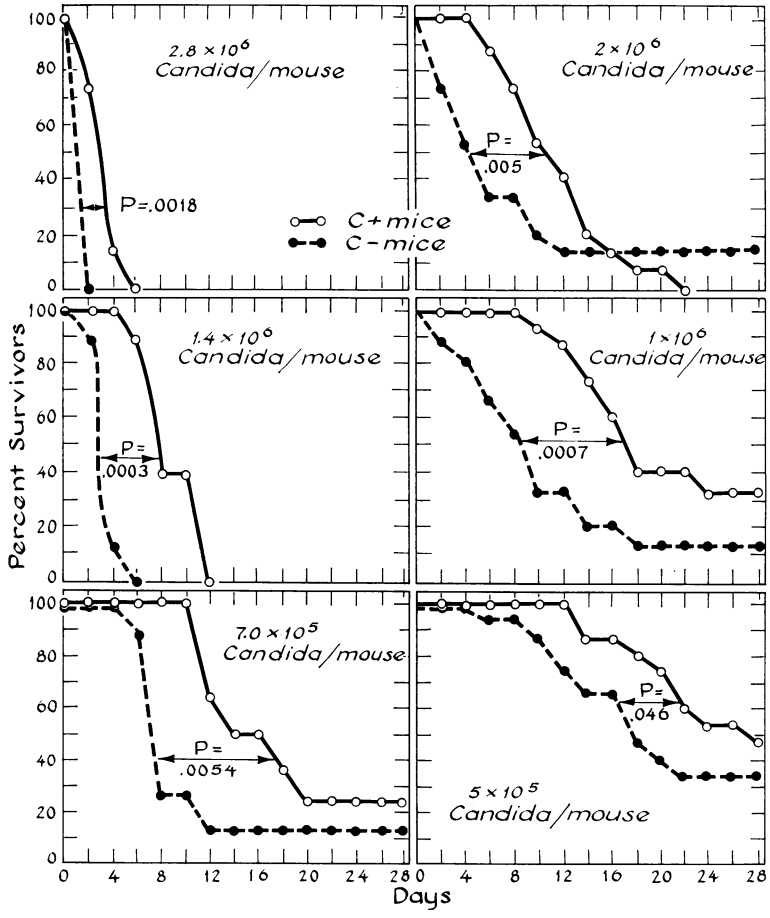

FIG. 1. *Survival of C+ and C− CF-1 female mice injected iv with different doses of Candida albicans. Groups of eight mice were used in the experiment shown on the left. Groups of 15 mice were used in the experiment shown on the right.*

to shed any light on possible mechanisms responsible for the effects seen in vivo. Lehrer and Cline (6) working with human serum failed to show any role played by heat-labile factors in the killing of *C. albicans* by polymorphonuclear leukocytes. A recent report presents evidence that C5 does enhance phagocytosis of yeast cells when PMNs are used as the phagocytes (10).

We have carried out many tests with the strain of *Candida* used for infecting mice and failed to show killing of yeast in vitro by antibody and mouse or guinea pig complement. Phagocytosis and digestion were not detectably different in the presence of complement from "normal" or C5-deficient mice. We have not yet compared phagocytic cells from "normal" and C5-deficient mice for capacity to ingest and digest yeast cells.

The following speculations are based on some facts about the pathogenesis of the disease and the nature of the infecting organism.

Candida cells when injected into animals cause reactions indicating that the cells are toxic (14). A substance similar to the endotoxin found in gram-negative bacteria was recovered from *Candida* by Isenberg et al. (4). Furthermore, Kobayashi and Friedman (5) by using phenol extraction were able to isolate an endotoxin-like fraction from *C. albicans* which was pyrogenic for rabbits. It is known that lipopolysaccharides which contain the endotoxic moiety of gram-negative cell walls interact with C (3). This interaction involves late acting components of C (C3–9). We speculate that this interaction may contribute to detoxifying the endotoxin. There is no persuasive evidence that endotoxin is the principal infection-promoting factor in candidiasis. Nevertheless, its endotoxic contents may contribute to the pathogenesis of the infection; if so, one can understand how animals possessing a system better able to inactivate the endotoxin would benefit therefrom.

Another mechanism of enhanced resistance by C^+ mice might occur somewhat as follows. It has been shown that the kidney is the organ most susceptible to infection after iv inoculation of mice with *C. albicans* (8). The yeast breaks into the renal tubular lumen and grows there, thereby perhaps evading somewhat the host defense mechanisms (7). Cellular reaction to invasion of the kidney of *Candida* is slow. A slight inflammatory response is seen only 8 hr after infection (7). It has been claimed that endotoxin incubated in C^+ but not in C^- mouse serum generates chemotactic activity (9). It may be that in C^+ mice the mobilization of phagocytic cells occurs sooner and to a greater extent than in C^- mice. Perhaps animals able to mobilize defense mechanisms more quickly are thereby able to survive the infection longer, which is the essence of our observation.

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