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Immediate Hypersensitivity to *Cryptococcus neoformans*

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The role of the capsular polysaccharide in anaphylactic reactions to *Cryptococcus neoformans* was investigated. Groups of mice were sensitized with viable cells of either a moderately encapsulated strain of *C. neoformans* or a non-encapsulated variant. Anaphylactic reactions were observed in both groups of mice to a similar extent when challenged with whole cells. Mice sensitized with the encapsulated strain and challenged with homologous polysaccharide showed only mild hypersensitivity symptoms. Mice sensitized with either the encapsulated or the non-encapsulated strain showed cross-reactivity when challenged with killed cells of the heterologous strain. These data indicate that the capsular polysaccharide plays a minor role in anaphylactic reactions to *C. neoformans* and that the sensitizing antigen is probably located in the cell wall of the yeast.

Allergic reactions, especially of the delayed type, are a common manifestation observed in diseases caused by fungi (15).

Immediate hypersensitivity reactions to yeast-like pathogens have been reported on relatively few occasions. A polysaccharide fraction from *Candida albicans* has been found to elicit an immediate reaction in asthmatic patients, as well as in guinea pigs previously sensitized to this organism (6). Guinea pigs passively sensitized with rabbit antiserum to *C. albicans* also developed anaphylactic shock when challenged with a polysaccharide-containing fraction from *C. albicans* (7). It has been reported that guinea pigs and mice passively sensitized with rabbit anti-cryptococcal antisera develop anaphylactic-like reactions upon challenge with cryptococcal polysaccharide (1). Further work on immediate hypersensitivity to *Cryptococcus neoformans* has not been reported.

The role of the capsular polysaccharide in the pathogenicity of *C. neoformans* is not completely understood. Cryptococcal capsular polysaccharide has been shown to inhibit phagocytosis (2, 3, 8, 11) and to induce immunological unresponsiveness in experimental animals (10, 14). Similarly, the role of antibodies in cryptococcosis is not clear. Circulating antibodies have been demonstrated in the sera of patients with cryptococcosis only during the recovery phase of the disease, when the antigen levels have declined. Circulating antibodies have also been found in patients with early or low-grade infection. These findings suggest that antibodies may play a role in the host recovery in cryptococcosis (13).

It was the purpose of this study (i) to further investigate hypersensitivity responses in mice actively sensitized with *C. neoformans* by means of a quantitative in vivo method for scoring anaphylaxis in mice and (ii) to elucidate the role of the capsular polysaccharide in these reactions.

The organisms utilized in this study were *C. neoformans* B3502(a), which is a virulent, encapsulated strain (serotype C), and *C. neoformans* 602, which is non-encapsulated. The characteristics of this latter strain have been described in detail elsewhere (9). This strain is closely related to serotype D, but lacks some antigenic determinants (9). In experiments where viable organisms were employed, the two strains were grown in Sabouraud dextrose broth at 37°C for 36 h and the cells were harvested by centrifugation and washed three times in sterile physiological saline, thus excluding the possibility of the reactions observed in mice due to components in the medium. Cell counts were determined with a hemacytometer and in all cases related very closely to viable plate counts. Formalin-killed cells were prepared by adding Formalin (3% final concentration) to 36-h Sabouraud dextrose broth cultures and further incubating at 37°C for 18 h. The cultures were checked for viability by streaking samples on Sabouraud dextrose agar plates, and the cells were finally washed three times in sterile physiological saline when sterility was ascertained.

Soluble capsular polysaccharide was extracted and purified from *C. neoformans* B3502(a). The organisms were grown in cryptococcal capsule broth, a synthetic medium previously reported to enhance capsule synthesis (12), for 4 to 5 days at 37°C. Cells were killed by the addition of Formalin (3% final concentration). The procedure followed for the extraction and purification of soluble polysaccharide was that of Farhi et al. (5) and Kozel and Cazin (9). Viable cells of both strains were used to sensitize and challenge CF-

1 female mice (Carworth Division of Charles River Breeding Laboratories, Inc., Wilmington, Mass.); in some of the experiments the purified capsular polysaccharide was also used for the challenge. Groups of mice were sensitized intraperitoneally (i.p.) with 10^4 viable cells of *C. neoformans* B3502(a) in sterile physiological saline. When *C. neoformans* 602 was used in sensitization and challenge of mice, then groups of animals were injected i.p. with 8×10^6 to 10^7 viable cells. At weekly intervals for a period of 5 weeks, groups of seven mice were challenged i.p. with 10^6 killed cells of the homologous strain and observed for symptoms of immediate hypersensitivity. In experiments where the purified capsular polysaccharide was used for the challenge, the mice were sensitized i.p. with 10^4 viable cells of strain B3502(a). Groups of seven mice were then challenged weekly i.p. with 0.5 or 1 mg of homologous polysaccharide. Studies on cross-hypersensitivity were performed by sensitizing groups of female Swiss albino mice (Simonsen Laboratories, Inc., Gilroy, Calif.) with *C. neoformans* B3502(a) or *C. neoformans* 602 and challenging different groups of these mice with the homologous and heterologous strains. All of the challenged mice were observed and scored for symptoms of anaphylaxis according to published methods (4, 17), as shown in Table 1.

Table 2 shows results of experiments in which groups of mice sensitized with viable cells of a moderately encapsulated strain [B3502(a)] or with viable cells of a non-encapsulated isolate (602) were challenged with killed cells of each strain. These experiments were initiated to obtain some evidence for the role of the capsular polysaccharide in immediate hypersensitivity to *C. neoformans*. Mice were observed and scored individually for symptoms of systemic anaphylaxis. The score given to each individual mouse represents the relative individual score. The relative individual scores for all mice in a group were added to give the total relative group score. Mice challenged with whole cells of either the encapsulated or the non-encapsulated strain showed hypersensitivity symptoms to a similar extent when challenged at the same times after sensitization. In both groups of mice, anaphylactic reactions were higher when challenge was done 22 days after sensitization; total relative group scores assigned were 17 and 16 for the encapsulated and the non-encapsulated strain, respectively. In all cases, mice showed complete recovery by 1 h.

TABLE 1. *Method for scoring symptoms of anaphylaxis*

Relative individual score	Symptoms
0	No observable change in activity
1	Slight change: hyperactivity; hypersensitivity with respect to touch and noise; hyperventilation; scratching
2	Hunched with ruffled fur; will move upon stimulation; partial paralysis of rear legs; shallow breathing
3	Head stretched forward and often to the side; in prone position; gasping and irregular breathing; convulsions
4	Death

TABLE 2. *Immediate hypersensitivity symptoms in mice sensitized with C. neoformans B3502(a) and C. neoformans 602*[a]

Time of challenge after sensitization (days)	*C. neoformans* B3502(a)		*C. neoformans* 602	
	RIS[b]	TRGS[c]	RIS	TRGS
7	1, 0, 2, 1, 1, 2, 2	9	1, 1, 1, 0, 1, 1, 1	6
14	2, 2, 2, 1, 1, 1, 1	10	1, 2, 2, 2, 1, 2, 2	12
22	2, 2, 3, 3, 3, 2, 2	17	2, 3, 2, 2, 3, 2, 2	16
29	2, 2, 1, 1, 2, 2, 2	12	2, 2, 1, 2, 3, 2, 2	14
36	1, 1, 0, 1, 0, 1, 1	5	1, 1, 0, 1, 1, 1, 1	6

[a] Seven mice were used in each group. All mice recovered by 1 h.

[b] RIS, Relative individual score 30 min after challenge.

[c] TRGS, Total relative group score: sum of relative individual scores at 30 min for all mice in a group.

It has been previously reported that mice passively sensitized with rabbit anticryptococcal antisera developed anaphylactic-like reactions upon immediate intravenous challenge with cryptococcal polysaccharide (1). In this study, mice actively sensitized with viable cells were challenged with homologous polysaccharide at 1, 2, and 3 weeks after sensitization. This group of mice showed only mild hypersensitivity symptoms (Table 3). In no case did mice show hypersensitivity reactions to which an individual score higher than 1 was given. The differences in hypersensitivity shown by this group of mice and the groups of mice challenged with whole cells were found to be statistically significant (F test and Q test) (16). Control mice, that is, mice receiving sterile physiological saline in the sensitizing dose, showed no symptoms of immediate hypersensitivity when challenged with either whole cells or polysaccharide.

The objective of studying cross-hypersensitivity reactions was to further evaluate the role played by cryptococcal polysaccharide in im-

mediate hypersensitivity to *C. neoformans*. If the capsular polysaccharide is involved in hypersensitive reactions, mice sensitized with the non-encapsulated strain would not be expected to show symptoms of hypersensitivity when challenged with the encapsulated strain. In the first experiment, mice sensitized with viable cells of the encapsulated strain were challenged 3 weeks later with killed cells of the homologous strain or with killed cells of the non-encapsulated strain. In the second experiment, mice sensitized with viable cells of the non-encapsulated strain were challenged with killed cells of the homologous strain or with killed cells of the encapsulated strain. The results obtained in this study are shown in Table 4. Mice sensitized with the non-encapsulated strain showed cross-hypersensitivity reactions when challenged with the encapsulated strain. Hypersensitive reactions also developed in mice sensitized with the encapsulated strain when challenged with either the encapsulated or the non-encapsulated strain. Hypersensitivity symptoms developed in both groups of mice to a similar extent.

TABLE 3. *Immediate hypersensitivity symptoms in mice sensitized with viable cells of C. neoformans B3502(a) and challenged with homologous polysaccharide*

Time of challenge after sensitization (days)	Score after challenge[a]	
	RIS[b]	TRGS[c]
7	0, 0, 0, 1, 1, 0, 1	3
14	1, 0, 0, 0, 1, 1, 0	3
22	1, 0, 0, 1, 1, 0, 0	3

[a] Challenge with 1 mg of cryptococcal polysaccharide.
[b] RIS, Relative individual score. Each number represents one mouse.
[c] TRGS, Total relative group score.

TABLE 4. *Immediate hypersensitivity symptoms in mice sensitized with viable cells of C. neoformans B3502(a) or 602 and challenged with the homologous or heterologous strain*

Sensitizing strain	Score with challenge strain:			
	B3502(a)[a]		602[b]	
	RIS[c]	TRGS[d]	RIS	TRGS
B3502(a)[e]	1, 1, 2, 2, 3	9	1, 2, 3, 1, 2	9
602[f]	1, 2, 2, 3, 3	11	1, 3, 3, 3, 2	12

[a] Mice were challenged with 10^6 killed cells i.p. 3 weeks after sensitization.
[b] Mice were challenged with 10^7 killed cells i.p. 3 weeks after sensitization.
[c] RIS, Relative individual score.
[d] TRGS, Total relative group score.
[e] Mice received 5×10^4 viable cells i.p.
[f] Mice received 5×10^6 to 6×10^6 viable cells i.p.

Experiments are in progress in an attempt to elucidate the role of cell wall and cytoplasmic antigens extracted from various strains of *C. neoformans* in immediate hypersensitivity.

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