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Cryptococcus neoformans Galactoxylomannan Contains an Epitope(s) That Is Cross-Reactive with *Aspergillus* Galactomannan

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We report a case of cryptococcosis in which a serum enzyme-linked immunosorbent assay (ELISA) for *Aspergillus* galactomannan was positive, with no evidence of aspergillosis. Soluble antigens from 19 *Cryptococcus neoformans* strains and purified carbohydrates of *C. neoformans* capsule were thus assayed in the *Aspergillus* galactomannan ELISA. Antigens from all *C. neoformans* strains, and purified galactoxylomannan, gave a positive reaction, suggesting that *C. neoformans* galactoxylomannan contains an epitope(s) that is cross-reactive with *Aspergillus* galactomannan.

Cryptococcus neoformans is a basidiomycetous encapsulated yeast that grows on soils in association with bird excreta or certain trees. In immunocompromised individuals, especially in patients with AIDS, the most common presentation of cryptococcal infection is a meningoencephalitis. Four major serotypes, A, B, C, and D, are identified based on the antigenic structure of the polysaccharide capsule. Serotype A is responsible for the majority of infections in AIDS patients. The capsule is composed of two polysaccharides (1, 3). The glucuronoxylomannan (GXM) accounts for about 90% of the capsule mass, governs serotype specificity, and is a prominent virulence factor. The galactoxylomannan (GalXM) is a minor component and makes up about 7% of the capsular mass. Shedding of GXM occurs in body fluids and infected tissues during cryptococcal infection (5). The diagnosis of cryptococcosis is based on culture of *C. neoformans* from cerebrospinal fluid (CSF), blood, and various biological fluids or tissue samples and on detection of capsular antigen in serum and/or CSF. We observed a patient with pulmonary cryptococcosis whose serum was positive by the Platelia *Aspergillus* assay, a commercially available enzyme-linked immunosorbent assay (ELISA) for the soluble *Aspergillus* galactomannan antigen (8), but who exhibited no additional evidence of aspergillosis. We hypothesized that soluble components from *C. neoformans* shed during infection may react in the Platelia *Aspergillus* assay and present experimental data to support this hypothesis.

The patient, a 43-year-old man, was admitted with weight loss, fever (39°C), dyspnea, cough, and hemoptysis. Human immunodeficiency virus-specific antibodies were detected with both the ELISA and Western blot assays. Other laboratory findings included a CD4 cell count of 6/mm^3 and a human immunodeficiency virus viral load of 6.07 log RNA/ml. Chest roentgenogram and computed tomographic scan showed infiltration of the lower left lobe and multiple nodules within both lungs. Bronchoscopic examination revealed an obstructive hemorrhagic lesion of the lower left lobar bronchus. A biopsy was performed, and epinephrine lavages were repeated to stop hemoptysis. Because of unexplained fever and lung infiltrate, amoxicillin-clavulanate was administered. Fluconazole (200 mg/day) was also started because of oral candidiasis. Clinical status did not improve. On day 6, a blood culture (Bactec 9240; Becton Dickinson) was positive and showed capsulated yeasts identified as *C. neoformans* (API 32C; Biomérieux, France). The cryptococcal antigen titer in serum samples collected on days 7 and 8 was 1:20 (Calas; Meridian Bioscience). The Platelia *Aspergillus* reactivity indexes for serum samples collected on days 5, 8, and 9 were 4, 0.5, and 2.2, respectively (Bio-Rad, Marnes-La-Coquette, France). Cryptococcal antigen detection, direct microscopic examination, and culture of the CSF were negative. Cultures of sputum grew *C. neoformans*. Histopathological examination of pulmonary biopsy revealed chorionic invasion with Gomori-Grocott-positive capsulated yeasts consistent with *C. neoformans*, but no hyphae were observed. None of the respiratory, CSF, and blood cultures grew *Aspergillus* spp. Intravenous fluconazole (800 mg/day) and amphotericin B deoxycholate (1 mg/kg of body weight/day) were started on day 6. Blood cultures remained negative thereafter. The patient died 14 days later with septic shock and acute respiratory distress of unexplained cause.

We found no satisfactory explanation for the positive Platelia *Aspergillus* assay result for this patient. Although *Aspergillus*-PCR was not performed on the pulmonary biopsy, the absence of filaments at histopathological examination and the negativity of *Aspergillus* cultures made coinfection by *C. neoformans* and *Aspergillus* spp. very unlikely. The patient had no clinical signs suggestive of an extrapulmonary aspergillosis. However, we cannot formally exclude the possibility of a cryptic locus of infection. The patient received amoxicillin-clavulanate, a treatment that was recently suggested to cause false positivity of the Platelia *Aspergillus* assay (11). However, this

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TABLE 1. Index of reactivity of heat-treated and not treated soluble antigens from suspensions of clinical and reference strains of *Cryptococcus* spp. in the Platelia *Aspergillus* assay[a]

Strain designation	Site of isolation	Identification	Index of reactivity in expt:		
			1 (no heat treatment)	2 (no heat treatment)	3 (heat treatment)
44	CSF	*C. neoformans*	4.8	>5	3.4
101	Blood culture	*C. neoformans*	4.7	>5	3.1
294	Blood culture	*C. neoformans*	>5	>5	>5
545	CSF	*C. neoformans*	3.8	>5	3.3
590	Urine	*C. neoformans* serotype A	>5	>5	>5
648	Blood culture	*C. neoformans* serotype A	4.1	>5	>5
681	CSF	*C. neoformans* serotype A	3.6	>5	>5
861	CSF	*C. neoformans*	4.8	>5	3.4
1451	CSF	*Cryptococcus sp.*	5	>5	3.4
1782	BAL	*C. neoformans* serotype D	>5	>5	>5
1829	Blood culture	*C. neoformans* serotype A	3.5	>5	>5
2323	Toe nail	*C. laurentii*	>5	>5	>5
2500[b]	Blood culture	*C. neoformans* serotype A	4.9	>5	3.5
2501[b]	Sputum	*C. neoformans* serotype A	4	>5	>5
NIH 68	Reference strain	*C. neoformans* serotype A	ND	3.1	2.6
NIH 112B	Reference strain	*C. neoformans* serotype B	ND	>5	>5
NIH 52D	Reference strain	*C. neoformans* serotype D	ND	2.8	2.2
CDC B.238	Reference strain	*C. neoformans* serotype C	ND	>5	>5
Cap 67	Reference strain	*C. neoformans* non typable	ND	>5	3.5

[a] CSF, cerebrospinal fluid; BAL, bronchoalveolar lavage; ND, not done. Experiments 2 and 3 were performed on the same antigenic extracts.
[b] Isolates 2500 and 2501 were obtained from the patient described in this paper.

information was not available at the time our patient was treated. Besides, this possibility does not change the overall conclusion of the experimental protocol below.

We thus hypothesized that the Platelia *Aspergillus* assay positivity may have been due to cross-reactive antigens released by *C. neoformans*. To test this possibility, soluble antigens from a panel of clinical and reference strains of *Cryptococcus* spp. (Table 1) were tested for their reactivity in the Platelia *Aspergillus* assay. Cultures were plated on malt agar and incubated at 30°C for 5 days. For each strain, 5 to 10 yeast colonies were suspended in 1 ml distilled water. After vigorous agitation for 1 min, the suspensions were centrifuged for 5 min at 10,000 × *g*, and the supernatants were collected for antigen detection. Two batches were prepared from independent cultures of each strain. Cryptococcal antigen detection was performed with the cryptococcal antigen latex agglutination system according to the instructions of the manufacturer, without pronase pretreatment. *Aspergillus* galactomannan detection was performed with the Platelia *Aspergillus* test, a sandwich immunoenzymatic assay based on a monoclonal antibody that binds *Aspergillus* galactomannan (Fig. 1 [adapted from reference 9]). The first batch of culture supernatants was tested without prior heating (Table 1) (experiment 1). The second batch of supernatants was tested with or without prior heating at 100°C (Table 1) (experiments 2 and 3). Heating is recommended by the manufacturer to precipitate proteins that may interfere

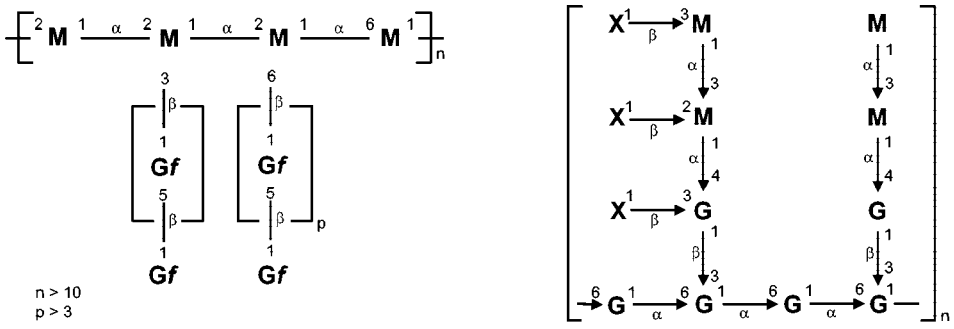

FIG. 1. Proposed structures of the *Aspergillus fumigatus* galactomannan (adapted from references 8 and 9 with permission) and the *Cryptococcus neoformans* galactoxylomannan (adapted from reference 1 with permission). The repeating unit of the galactomannan secreted by *Aspergillus fumigatus* consists of a linear core of mannan residues with α-1,2- and α-1,6-linked residues. Antigenic side chains composed exclusively of β-1,5 galactofuranosyl units are linked to the C-6 and C-3 positions of the α-1,2-linked mannose units of the core. These side chains have an average degree of polymerization of 4 and are the target of the antigalactomannan monoclonal antibody employed in the Platelia *Aspergillus* test. The repeating unit of the *Cryptococcus neoformans* galactoxylomannan consists of a backbone of α-1,6-linked galactose units, with side chains composed of galactose, mannose, xylose, and *O*-acetyl residues branched on alternate galactose residues. Small quantities of galactofuranose are present in the GalXM in some, but not all, *C. neoformans* isolates (6). The linkage of these galactofuranose units has not been characterized (14). M, mannan; G, galactose; Gf, galactofuranose; X, xylose.

TABLE 2. Index of reactivity of heat-treated and not treated purified components of the *C. neoformans* capsule in the Platelia *Aspergillus* assay[a]

Concn of carbohydrate	Index of reactivity					
	GXM serotype A		GXM serotype D		GalXM	
	No heat	Heat	No heat	Heat	No heat	Heat
10 μg/ml	0.14	0.07	0.13	0.06	>5	>5
1 μg/ml	0.14	0.05	0.11	0.07	>5	3.4
100 ng/ml	0.09	0.05	0.12	0.03	1.25	0.39
10 ng/ml	0.11	0.04	0.19	0.04	0.33	0.08
1 ng/ml	0.12	0.05	0.19	0.07	0.13	0.06

[a] "No heat" and "heat" indicate that there was not and was heat treatment prior to the enzyme immunoassay, respectively.

with the immunoenzymatic reaction. Each series contained several controls: distilled water and a negative serum sample as negative controls, a positive-control serum sample, and a standard serum sample containing galactomannan at 1 ng/ml provided by the manufacturer. For each sample, the index is the ratio of the optical density of the sample to the optical density of the standard serum. In clinical practice, an index value of ≥1.5 is indicative of a positive reaction, an index of >1 and <1.5 is intermediate, and an index of <1 is considered nonsignificant. However, it was recently suggested that a cutoff value at 0.5 was clinically relevant (10).

Serial dilutions of the supernatant from isolate 2500 were first assayed in both antigen detection tests. The ELISA galactomannan index decreased from 0.977 to 0.142, with dilutions of the supernatant providing a cryptococcal antigen titer decreasing from 1:20 to 1:2. When the cryptococcal antigen titer was >1:200, the ELISA galactomannan index was >5. Thus, soluble antigens from *C. neoformans* reacted in the *Aspergillus* galactomannan test. Reactivity was dilution dependent and paralleled cryptococcal antigen titer. An identical approach was applied to other clinical isolates and reference strains of *Cryptococcus* spp. Based on the first experiment, dilutions of supernatants corresponding to a cryptococcal antigen titer of 1:20 or more were assayed in the *Aspergillus* galactomannan ELISA. Table 1 shows that the capsule of *C. neoformans* serotypes A, B, C, and D, as well as *Cryptococcus laurentii*, released antigens reactive in the Platelia *Aspergillus* assay.

To identify which of the *C. neoformans* antigens was reactive in the *Aspergillus* galactomannan ELISA, purified components from the capsule (GXM serotype A and serotype D and GalXM, a gift from R. Cherniak, Georgia State University, Atlanta, GA) were used (2, 6). The carbohydrates were diluted in sterile distilled water prior to utilization. Solutions at 10 μg/ml of each purified carbohydrate reacted in the cryptococcal antigen detection test. Table 2 shows that GalXM was the capsular component reactive in the *Aspergillus* galactomannan assay. This reactivity was dose dependent and specific since GXM showed no reactivity. These observations are consistent with the reactivity of Cap67 (Table 1), a mutant strain of *C. neoformans* that lacks GXM (1). The GalXM of *C. neoformans* consists of a backbone of galactose units, with side chains composed of galactose, mannose, xylose, and *O*-acetyl residues branched on alternate galactose residues (Fig. 1 [adapted from reference 1]). GalXMs from different *C. neoformans* serotypes differ in anomeric linkages between sugar residues (3). Despite these variations, the GalXM epitope recognized by the *Aspergillus* galactomannan monoclonal antibody is apparently conserved in all *C. neoformans* serotypes and strains (Table 1).

Whether or not GalXM is released during cryptococcal infection is unclear. In a single study, serum samples from patients with cryptococcosis reacted with GalXM, but only 22% had antibody titers above the threshold of positivity (12). Moreover, antibody production per se does not provide definite evidence that shedding of GalXM occurs, since epitopes exposed at the surfaces of *Cryptococcus* cells may well elicit an antibody response. Taken together, clinical and experimental data reported herein indicate that shedding of GalXM may occur in patients with cryptococcosis. The reactivities of *Aspergillus* galactomannan assays based on the EB-A2 monoclonal antibody in either the ELISA or the latex agglutination format have been reported for other fungi (7, 13), food (4), and drugs such as cyclophosphamide, and piperacillin-tazobactam (15). This is the first study reporting cross-reactivity with cryptococcal antigens (7, 13). Amoxicillin-clavulanate may also cause a positive Platelia *Aspergillus* assay result (11), and if this is confirmed, the possibility of a role for this treatment in the positivity of the test for our patient cannot be excluded. However, the experimental protocol described clearly demonstrates that *C. neoformans* GalXM contains an epitope(s) that is cross-reactive with *Aspergillus* galactomannan and may thus be the cause for a positive *Aspergillus* galactomannan ELISA result.

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