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Cryptococcal Glucoxylomannan Does Not Exhibit Cross-Reactivity in the MVista *Histoplasma* Antigen Enzyme Immunoassay▽

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The potential for cross-reaction between *Cryptococcus neoformans* and *Histoplasma capsulatum* in antigen assays was evaluated. We tested patient samples, spleens from infected mice, and purified polysaccharides in the MVista *Histoplasma* antigen enzyme immunoassay and cryptococcal antigen latex agglutination system for cross-reactivity, and none was observed.

The diagnosis of invasive or systemic mycosis is often accomplished through the detection of polysaccharide antigens in body fluids by immunologic assays. The specific polysaccharides detected are galactomannans (GalM) of *Aspergillus* spp. (2) and *Histoplasma capsulatum* (1) and glucuronoxylomannan (GXM) of *Cryptococcus neoformans* (2). We have observed several cases in which tests for both cryptococcal GXM and *Histoplasma* GalM were positive and where dual infections were documented. However, the literature on dual infection is not extensive (3). This prompted us to investigate further the presence of cross-reactivity between cryptococcal GXM and *Histoplasma* GalM.

Cryptococcal antigen latex agglutination system (CALAS) kits were purchased from Meridian Bioscience (Cincinnati, OH) and used according to the manufacturer's instructions. The serum samples were treated with pronase before being tested with a CALAS kit. The MVista *Histoplasma* antigen enzyme immunoassay (EIA) was performed at MiraVista Diagnostics (Indianapolis, IN) as previously reported (1).

Histoplasma and *Cryptococcus* polysaccharide antigens were prepared and purified as previously described (1, 2). *Histoplasma* GalM and cryptococcal GXM, galactoxylomannan, and capsular polysaccharide were tested at a concentration of 1 μg/ml, and the CALAS kit positive control was tested undiluted. Cryptococcal GXM, galactoxylomannan, and capsular polysaccharide and the CALAS control were strongly positive (agglutination score of 3+ or 4+) by CALAS assay but negative in the *Histoplasma* antigen EIA. Conversely, *Histoplasma* GalM was positive in the *Histoplasma* antigen EIA (>39 ng/ml) but negative by CALAS assay.

Twenty-nine residual serum specimens from 15 patients with progressive disseminated histoplasmosis were tested by CALAS assay, in a study approved by the institutional review board of Clarian Hospital, Indianapolis, IN. All had underlying immunosuppression, including AIDS in 10, organ transplantation in 2, and miscellaneous causes in 3 patients. The basis for diagnosis of histoplasmosis was *Histoplasma* antigenemia and antigenuria in seven cases, culture in six cases, and histopathology in two cases. *Histoplasma* antigenemia ranged from <0.6 to >39 ng/ml (median, 2.5 ng/ml). No samples were positive by CALAS assay (Table 1).

Residual serum or cerebrospinal fluid samples from 25 patients with cryptococcosis were previously described (2). The CALAS assay was positive for 24 of 25 patients, at titers of 1:1 to 1:65,536 (median, 1:1,024). Culture was the basis for diagnosis for the 25th patient. None of the samples were positive in the *Histoplasma* antigen EIA (Table 1).

In a previously described murine histoplasmosis model (4), eight B6C3F1 mice were infected intranasally with 1×10^6 *H. capsulatum* organisms and euthanized on day 10 of infection according to institutional guidelines (4). Spleen tissue was homogenized in 2 ml of RPMI medium and tested in the *Histoplasma* antigen EIA following 10-fold dilution but used undiluted in the CALAS assay. Spleen tissues contained high levels of *Histoplasma* antigen but were negative by CALAS assay (Table 1).

In a cryptococcal experimental infection model (2), 12

TABLE 1. Cross-reactivity in clinical specimens and experimental infection

Sample type	No. of positive samples/total no. of samples (% positive, 95% confidence interval)	
	Histoplasma antigen EIA	CALAS assay
Histoplasmosis samples		
Human serum	29/29 (100, 88.3–100)	0/29 (0, 0–11.7)[b]
Murine spleen[a]	8/8 (100, 67.6–100)	0/8 (0, 0–32.4)[b]
Cryptococcosis samples		
Human serum or cerebrospinal fluid	0/25 (0, 0–13.3)	24/25 (96, 80.5–99.3)[b]
Murine spleen[a]	0/12 (0, 0–24.2)	12/12 (100, 75.8–100)[b]

[a] Spleen tissues from mice infected with *H. capsulatum* were tested in the *Histoplasma* antigen EIA at a 1:10 dilution and were used undiluted in the CALAS assay. Spleen tissues of mice with cryptococcosis were tested undiluted.
[b] $P < 0.001$ for comparison of *Histoplasma* antigen EIA and CALAS assay, using Fisher's exact test.

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BALB/c mice were infected intravenously with 1×10^4 *C. neoformans* strain 24067 organisms and then euthanized on day 7 of infection. The spleen tissue was homogenized in 2 ml of RPMI medium and tested undiluted in the *Histoplasma* antigen EIA and the CALAS assay. Spleen tissues were positive by CALAS assay but negative in the *Histoplasma* antigen EIA.

In conclusion, no cross-reactivity between *Histoplasma* GalM and cryptococcal polysaccharides was observed. When both the CALAS assay and MVista *Histoplasma* antigen EIA are positive, dual infection should be suspected. Note that these findings should not be extrapolated to other *Histoplasma* or cryptococcal antigen assays.

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