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Isolation of a New Soluble Antigen from the Yeast Phase of *Histoplasma capsulatum*

MICHAEL W. REEVES, LEO PINE, LEO KAUFMAN, AND DAVID McLAUGHLIN

Research and Development Unit, Biological Reagents Section and Fungus Immunology Unit, Mycology Section, Center for Disease Control, Health Services and Mental Health Administration, Atlanta, Georgia 30333

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A method is described by which a soluble antigen was prepared from the yeast phase of *Histoplasma capsulatum*. This soluble preparation had a specificity greater than that of whole-cell yeast-phase antigens. In complement fixation tests with sera from human cases of histoplasmosis, blastomycosis, and coccidioidomycosis, the soluble antigen reacted in 12.1% of 141 tests with heterologous sera, whereas conventional whole-cell yeast antigens reacted in 47.3% of 91 tests with heterologous sera. The reactivities of the two types of antigens with homologous sera were essentially the same.

The complement fixation (CF) test is widely used in the diagnosis of human cases of histoplasmosis (5). The *Histoplasma capsulatum* antigens most frequently used in this test are histoplasmin (a broth filtrate from mycelial cultures) and saline suspensions of whole yeast-phase cells; however, both of these types of antigens are known to cross-react in varying degrees with antibodies to *Blastomyces dermatitidis* and *Coccidioides immitis* (2) and to other fungi (4).

Because a high-titered antiserum to the yeast phase of *H. capsulatum* may be indicative of an acute infection (3), numerous attempts have been made to extract an antigen from *H. capsulatum* yeast cells which would have the specificity and sensitivity needed to provide a reliable diagnosis of histoplasmosis. The antigens described thus far, however, have tended to be either insensitive (10, 12), unstable (8), or nonspecific (1). Certain soluble yeast-phase antigens described as specific and sensitive have been reported (6, 11), but they have not come into general use, possibly because of the complex isolation procedures involved in their preparation or because other investigators have been unable to duplicate these antigens (8). The purpose of this note is to report a soluble antigen of relatively high specificity and sensitivity which has been isolated by a simple procedure from the yeast phase cells of *H. capsulatum*.

A standard CF antigen (13) consisting of a 10% (v/v) suspension of whole yeast cells of *H. capsulatum* strain 6623 (7) in saline containing 1:10,000 Merthiolate was found to be anticomplementary (AC) when tested after storage at 4 C for 3 months. During attempts to locate the source of the AC activity, it was noted that the supernatant fluid of the cell suspension had a low level of CF activity with human sera from cases of histoplasmosis but was devoid of AC activity. Recognizing this as a possible source for a soluble *H. capsulatum* CF antigen, we concentrated the supernatant fluid 20-fold with Carbowax (polyethylene glycol, 20 M; Union Carbide Corp.) and dialyzed it against 50 volumes of 0.01 M phosphate-buffered saline, *p*H 7.0, containing 0.02% sodium azide. This preparation, designated lot 1, was found to have an optimal antigen dilution (OAD) of 1:64 in tests with homologous human sera. The yeast cells were then resuspended in the saline solution with Merthiolate to one-half the volume of the original whole-cell antigen. This suspension was held at 4 C for a week, and then the supernatant fluid was removed by centrifugation and the cells were resuspended again to the same volume. This process was repeated four times. The supernatant fluids were pooled, concentrated 10-fold, and dialyzed as before. Although this second preparation had been made from the same cells as lot 1, it was designated as lot 2 because of the differences in extraction and concentration. It had an OAD of 1:16. A second whole-cell suspension of *H. capsulatum* strain 6623 yeast phase was prepared by the same method as the first (13) and

was stored at 4 C for 4 weeks (this suspension was devoid of AC activity). The supernatant fluid was then removed, concentrated 27-fold, and dialyzed. This preparation was designated lot 3 and was found to have an OAD of 1:128.

TABLE 1. *Complement fixation titers[a] obtained with human sera from proven cases of histoplasmosis*

Serum no.	Whole-cell antigens		Soluble antigens		
	Lot 43	Lot 45	Lot 1	Lot 2	Lot 3
43	64	32	64	64	64
74	64	32	128	64	64
197	64	32	32	32	32
415	64	64	32	32	32
607	16	32	8	8	8
722	64	32	64	64	64
1008	64	64	64	64	64
1021	64	32	32	32	16
1160	64	32	64	64	128
1163	32	32	64	64	64
1447	32	32	128	128	128
1552	8	8	8	8	0[b]
1602	64	32	64	64	64
1975	32	ND[c]	32	32	ND
2016	8	0	8	8	0
2263	64	64	64	64	64
2373	32	ND	32	32	ND
2424	64	64	64	64	64
2568	64	32	64	64	64
2798	64	64	128	64	128
2817	8	8	8	8	0
3845	32	ND	64	128	ND
4076	64	64	64	64	64
5945	128	32	128	64	32
6756	128	64	64	64	64

[a] End point dilution factors.
[b] Serum titer less than 8.
[c] Not done.

All three lots of soluble antigen were devoid of AC activity. The three soluble antigens and two whole-cell *H. capsulatum* yeast antigens (lot 43, OAD 1:64; and lot 45, OAD 1:32), taken from the inventory of the Biological Reagents Section, Center for Disease Control, were tested in parallel by CF (9) at their optimal antigen dilutions against human sera from culturally or histologically proven cases of histoplasmosis, blastomycosis, and coccidioidomycosis.

With all but a few of the sera, the whole-cell and soluble antigens demonstrated comparable sensitivity when tested with the homologous antisera (Table 1). Studies with the heterologous case sera (Table 2) revealed that the soluble antigens were more specific than the whole-cell antigens. The three soluble antigens gave 17 (12.1%) positive reactions out of 141 tests with heterologous sera, whereas the whole-cell antigens gave 43 (47.3%) positive reactions out of 91 tests. The degree of cross-reactivity seemed to vary with each lot of antigen. The fact that the soluble lots 1 and 2 seemed to be less cross-reactive than lot 3 with blastomycosis case sera (Table 2) suggests that the degree of cross-reactivity that is to be expected with soluble antigens of this type is influenced more by differences in the batches of cells the antigens are prepared from than by the number of times the cells are extracted or the degree of concentration. Since the soluble antigen could be recovered from cells which were not AC (lot 3), AC activity apparently is not related to the release of the soluble antigen.

Since all of the soluble antigens used in these tests were made with *H. capsulatum* strain 6623, a fourth antigen was prepared from the supernatant fluid (concentrated 20-fold) of a whole-cell yeast antigen of *H. capsulatum*

TABLE 2. *Complement fixation results obtained with human sera from proven cases of blastomycosis and coccidioidomycosis*

Antigen	Blastomycosis case sera		Coccidioidomycosis case sera	
	No. positive/ no. tested[a]	Cross-reactions (%)	No. positive/ no. tested	Cross-reactions (%)
Whole cell				
Lot 43	9/24	37.5	16/26	61.5
Lot 45	11/20	55.0	7/21	33.3
Soluble				
Lot 1	3/24	12.5	3/26	11.5
Lot 2	2/24	8.3	2/26	7.7
Lot 3	5/20	25.0	2/21	9.5

[a] A test was considered positive if an antigen reacted with a serum diluted 1:8 or greater. Only a few of these sera reacted at dilutions greater than 1:16.

strain A811 (lot 45) taken from the inventory of the Biological Reagents Section, Center for Disease Control. CF results with this preparation against homologous human sera showed that it was antigenic (OAD of 1:32) and devoid of AC activity. Because only a small volume of this preparation was available, it was not tested with heterologous human case sera; however, the results obtained with the homologous sera were sufficient to show that the release of soluble antigens under these conditions is not necessarily restricted to *H. capsulatum* strain 6623.

During these studies, it was observed that the OAD of lot 1 soluble antigen decreased from 1:64 to 1:8 when the antigen was stored at 4 C for 8 months. No loss in OAD titer was observed with lot 2, which was stored at 4 C for 6 months, or with lot 3, which was stored at 4 C for 4 weeks. The problem of the stability of this antigen and its physical, chemical, and immunological characteristics are presently under investigation. It is also considered important to determine if the method of extraction presented here may be applicable to the preparation of more specific soluble antigens from other pathogenic fungi. To our knowledge, this is the first time that a practical, soluble yeast-phase *H. capsulatum* antigen has been reported with this degree of specificity and stability. This antigen could prove to be useful in the development of a specific antigen for the diagnosis of histoplasmosis and for the production of specific diagnostic antisera.

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