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Evaluation of Cross-Reactions in *Histoplasma capsulatum* Serologic Tests

JOSEPH WHEAT,[1,2,3]* MORRIS L. V. FRENCH,[3] SUSAN KAMEL,[1,4] AND RAM P. TEWARI[5]

Indianapolis Veterans Administration Hospital,[1] Wishard Memorial Hospital,[2] and the Indiana University Medical Center,[3] Indianapolis, Indiana 46202; Indiana University, Bloomington, Indiana 47405[4]; and Southern Illinois University School of Medicine, Springfield, Illinois 62707[5]

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Cross-reactivity in *Histoplasma* serologic tests was evaluated by using sera from patients with histoplasmosis and other infections. Serum samples from 127 of 134 (95%) patients with histoplasmosis were judged positive by complement fixation tests, and 121 (90%) showed H bands, M bands, or both by immunodiffusion. Of these 134 patients, cross-reactions were seen to *Blastomyces dermatitidis* in 53 patients (40%), to *Coccidioides immitis* in 20 patients (16%), and to *Aspergillus fumigatus* in 3 patients (2%) by complement fixation. Serum samples from 5 of 99 patients (5%) with other fungal infections and from 5 of 46 patients (11%) with tuberculosis had M precipitin bands by the *Histoplasma* immunodiffusion test, whereas none of the 123 sera from patients with other bacterial, *Mycoplasma*, or viral infections showed H or M precipitin bands. In the complement fixation test, positive reactions were observed in 16 of 90 patients (18%) with other fungal infections, in 14 of 41 patients (34%) with tuberculosis, and in 18 of 105 patients (17%) with other bacterial, *Mycoplasma*, or viral infections. Positive reactions were seen by radioimmunoassay in 54 of 110 patients (49%) with other fungal infections, in 23 of 46 patients (50%) with tuberculosis, and in 35 of 123 patients (28%) with other bacterial, *Mycoplasma*, or viral infections. These results demonstrate a wider range of cross-reactions in *Histoplasma* serology than has been previously recognized, and the cross-reactivity was greatest when observed by radioimmunoassay. Caution should be exercised in the interpretation of serologic data from patients with suspected fungal infections.

Serologic tests have been used extensively for diagnosis and in epidemiologic studies of histoplasmosis (33, 44). The tests most commonly used in the serodiagnosis of histoplasmosis are the complement fixation (CF) and immunodiffusion (ID) tests. The use of these techniques has led to the detection of numerous otherwise unrecognized cases. However, uncertainty about the specificity of these tests has created serious problems in the diagnosis of systemic fungal infections.

Reported frequencies of false-positive results have ranged from 10 to 100% (11, 35). Factors which may explain this wide range of serologic cross-reactions include both the use of nonstandardized antigens and assays and the failure to exclude current or past histoplasmosis. Although cross-reacting antigens have been recognized among pathogenic fungi for several years, few comprehensive evaluations of cross-reactivity have been reported in which current immunologic reagents and procedures are used.

We previously reported the clinical usefulness of serologic tests in the diagnosis of histoplasmosis (41–45). These tests were highly sensitive and were positive in 90% of cases (43), including patients with culture-proven disseminated disease (41). The tests were also reasonably specific. However, those studies primarily included normal controls or controls with various bacterial infections. Few controls with other fungal diseases or tuberculosis, groups in which false-positive results would be expected to occur with greater frequency, were evaluated. Since our earlier studies were completed, we have gathered serum specimens from patients with either other fungal diseases or tuberculosis to be studied in a more thorough evaluation of cross-reactivity. In the present investigation, we determined the specificity and the range of cross-reactivity of the commercially available serologic tests and a newly described radioimmunoassay (RIA) for antibodies to *Histoplasma capsulatum*.

MATERIALS AND METHODS

Patient samples. (i) Bacterial, viral, and *Mycoplasma* infections. Serum samples from 17 patients with Legionnaires disease acquired in either Los Angeles, Calif., or England, from 10 patients with atypical pneumonia syndromes, from 15 subjects with *Mycoplasma* pneumonia, and from 10 subjects with respiratory syncytial virus infection were obtained during epidemiologic investigations by the Centers for Disease Control, Atlanta, Ga., and kindly supplied by W. Schleck (Public Health Laboratories, Halifax, Nova Scotia, Canada). Samples from 14 individuals from Illinois who seroconverted in the serogroup 1 *Legionella pneumophila* indirect fluorescent antibody assay were obtained from the Illinois Department of Health Laboratories. Serum samples were also tested from an additional 10 patients with Legionnaires disease, from 14 patients who seroconverted in the CF test for *M. pneumonia* antibodies, and from 37 patients with miscellaneous other pulmonary infections who were evaluated at our hospitals.

(ii) Fungal infections. Serum samples were available from 168 patients with histoplasmosis who were tested in our laboratories during the investigations of three outbreaks. These included 134 patients who were identified during two outbreaks in Indianapolis (43, 44) and 34 who were identified during an outbreak in Minnesota (J. N. Kuritsky, V. M. Thelen, M. L. Simpson, M. T. Osterkolm, R. J. Weeks, and M. J. Sprafka, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother. abstr. no. 1024, 1983). The speci-

* Corresponding author.

mens from the 134 patients who were identified during the Indianapolis outbreaks were selected for assessment of cross-reactivity with commercially available *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Aspergillus fumigatus* antigens by CF and ID. Patients diagnosed as having histoplasmosis during the Indianapolis outbreaks must have had an appropriate clinical illness, laboratory evidence for histoplasmosis, and no other identified cause for the illness. Laboratory criteria for histoplasmosis included the following: (i) positive culture or histopathologic demonstration of organisms consistent with *H. capsulatum* in tissues (proven cases), (ii) either CF titers of ≥1:32 to the yeast or to the mycelial antigens or fourfold titer changes (highly suggestive cases), or (iii) either CF titers of 1:8 or 1:16 to the yeasts or to the mycelial antigens or H or M precipitin bands by ID (presumptive cases) (22). Patients with highly suggestive or presumptive laboratory criteria were not considered to have histoplasmosis if other fungal pathogens or mycobacteria were identified by culturing and were thought to be the cause of the clinical syndrome. Furthermore, cases were excluded if they did not have a compatible clinical illness (45). The serum samples obtained from 34 patients who were infected during the histoplasmosis outbreak in Minnesota were obtained 6 weeks after exposure and were used in an assessment of the sensitivity of the different immunoassays in acute histoplasmosis, serving as a positive control group for analysis of cross-reactivity. A total of 46 serum samples were obtained from patients with fungal infections other than histoplasmosis who were evaluated at the Indiana University Medical Center Hospitals, and 52 others were kindly provided by Carol Kaufman, University of Michigan, Ann Arbor; Glenn Roberts, Mayo Clinic Foundation, Rochester, Minn.; Demosthenes Pappagianis, University of California, Davis; Jeffrey Jones, University of Wisconsin, Madison; George Sarosi, University of Texas Health Science Center, Houston; and Angela Restrepo, University of Antioquia, Medellin, Colombia. Histoplasmin skin tests were not performed on patients evaluated at our hospitals; however, skin test status is unknown for the patients who were evaluated elsewhere.

(iii) Mycobacterial infections. A total of 21 of the serum samples were from patients monitored at the Indiana University Medical Center Hospitals. Histoplasmin skin tests were not administered to these patients. The remaining 25 were obtained from Steven Kalish, Northwestern Medical Center, Chicago, Ill., and from the National Jewish Hospital Mycobacteriology Laboratory, Denver, Colo. The histoplasmin skin test status of these patients is unknown.

Serologic tests. The ID test was performed according to the instructions of the manufacturer with commercially available plates, *Histoplasma* ID antigen, and control sera (American MicroScan, Sacramento, Calif.) (45). Plates were incubated in moisture chambers at room temperature for 48 h. Results were read at 24 and 48 h. Any arcing of a positive control M or H band to carry it past a well containing a patient's serum was considered positive; other precipitin bands which did not form lines of identity with the positive control serum were disregarded.

CF tests were performed by using *Histoplasma* yeast, mycelial antigens, and positive control sera (American MicroScan). The *H. capsulatum* strain (CDC A811) used to prepare the yeast antigens for CF was the same as that used at the Centers for Disease Control. The mycelial antigens used in the CF test were prepared from mycelial-phase cultures of the two strains (GW and VC) used by the Veterans Administration reference laboratory. The mycelial strains used in the ID test were prepared from three soil isolates obtained from microfoci associated with histoplasmosis outbreaks in Kentucky. The mycelial antigens used for CF and ID were prepared in accordance with Centers for Disease Control procedures and were standardized by the manufacturer (American MicroScan) against reference antigens and control sera obtained from the Centers for Disease Control. Sheep erythrocytes and rabbit hemolysin were purchased from Flow Laboratories, Inc., McLean, Va. Guinea pig complement was obtained from Whittaker MA Bio-products, Walkersville, Md., and was rehydrated according to package directions. The CF test was performed according to the specifications described by the Centers for Disease Control. A 1:8 dilution of patient serum was the lowest dilution tested, and results of 1:8 or greater were considered positive, as has been recommended by others (22). Specimens from 20 patients with histoplasmosis were tested simultaneously at the Indiana State Board of Health and the Centers for Disease Control, and the results varied by no more than a single dilution in 18 of the 20 (unpublished data).

The solid-phase RIA was performed as previously described (44). Polystyrene tubes (12 by 75 mm) were coated with 0.2 ml of a 1:200 dilution of histoplasmin (American MicroScan) in 0.01 M Tris hydrochloride buffer (pH 7.0) at 37°C for 1 h. The tubes were incubated with 0.2 ml of 5% bovine serum albumin (BSA) in 0.1 M Tris hydrochloride (pH 7.0) for 1 h at 37°C to coat unoccupied binding sites, thereby preventing nonspecific attachment of the patient's serum or of radiolabeled antibodies added later. Next, 0.2 ml of the patient's serum which had been diluted 300-fold in 5% BSA in 0.1 M Tris-saline (pH 8.0) was incubated in the tube for 1 h at 37°C. Finally, immunoglobulin M (IgM) and IgG antibodies adherent to the solid-phase *H. capsulatum* antigens were measured with 0.2 ml of ^{125}I-labeled goat anti-human IgM or IgG at a concentration of approximately 2 μg/ml in 5% BSA in 0.10 M Tris-saline (pH 8.0); this last incubation was also conducted at 37°C for 1 h. The tubes were aspirated and rinsed three times between the first three steps and were washed five times after the last step. Each specimen was tested in triplicate. Results greater than 2.2 times the mean of 10 normal sera were considered positive, and the data were normalized so that the cutoff at 2.2 times the mean of the normal control sera equalled one RIA unit.

RESULTS

Specimens from 34 patients with histoplasmosis were obtained 6 weeks after a common, point-source exposure in Minnesota and were used in an objective comparison of the sensitivity of the different immunoassays in patients diagnosed as having histoplasmosis based on clinical findings alone. The ID test was positive for 22 of 34 subjects (65%), the CF test was positive for 29 of 34 subjects (85%), and the RIA was positive for 31 of 34 subjects (91%). Of the total of 110 patients with other types of fungal infections, 5 of 99 (5%) were judged positive by ID, 16 of 90 (18%) were judged positive by CF, and 54 of 110 (49%) were judged positive by RIA (Table 1). These 110 fungal controls included 23 with aspergillosis, 11 with blastomycosis, 30 with candidiasis, 21 with coccidioidomycosis, 8 with cryptococcosis, and 7 with paracoccidioidomycosis. Elevated levels of antibodies to *H. capsulatum* were observed in sera from patients with each type of fungal infection tested, with the highest frequency of positive judgments found for those samples with histo-

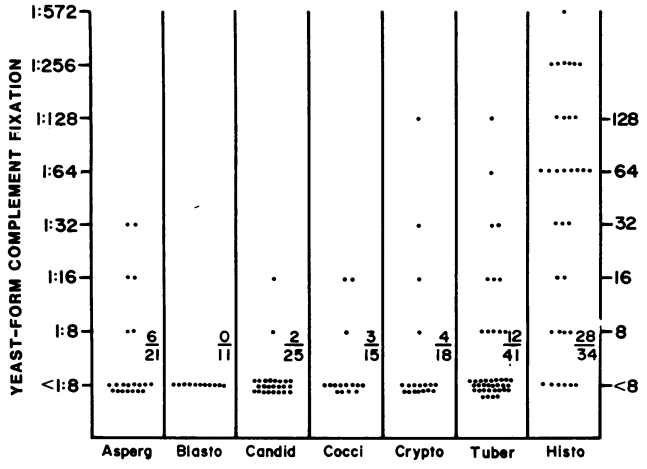

FIG. 1. Complement-fixing antibodies to the yeast form *H. capsulatum* antigen. Titers ≥1:8 are considered positive.

plasmosis infections and the highest false-positive rate found for those samples with aspergillosis (Fig. 1 to 4). Seropositivity was also common in patients with tuberculosis; the ID test was positive for 5 of 46 patients (11%), the complement fixation test was positive for 14 of 41 patients (34%), the IgM RIA was positive for 4 of 46 patients (9%), and the IgG RIA was positive for 23 of 46 patients (50%). Actual levels of cross-reactive antibodies in these controls were similar to those found in 34 individuals with histoplasmosis identified during the Minnesota outbreak (Fig. 1 to 4). The frequency of seropositivity in subjects with tuberculosis who were from Indianapolis, Ind., Chicago, Ill., and other parts of the United States was similar.

Cross-reactions occurred less frequently in other control groups. For 123 patients with a variety of other infections, *Histoplasma* antibodies were judged elevated by ID in none, elevated by CF in 18 of 105 patients (17%), and elevated by IgM RIA in 17 of 123 patients (14%) and by IgG RIA in 28 of 123 patients (23%) (Table 1). Samples from only one of the 18 patients (6%) judged positive by CF had titers of 1:32 or more, and samples from only 11 of the 123 (9%) were positive in the IgG RIA at levels of ≥2 RIA units. The infrequent seropositivity of patients with Legionnaires dis-

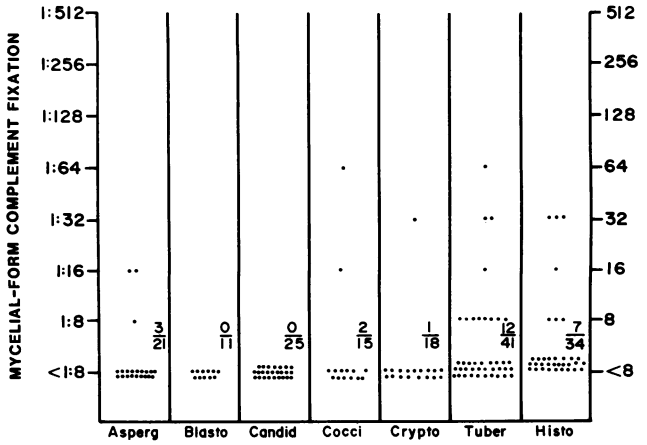

FIG. 2. Complement-fixing antibody titers to the mycelial form of *H. capsulatum* antigen. Titers ≥1:8 are considered positive.

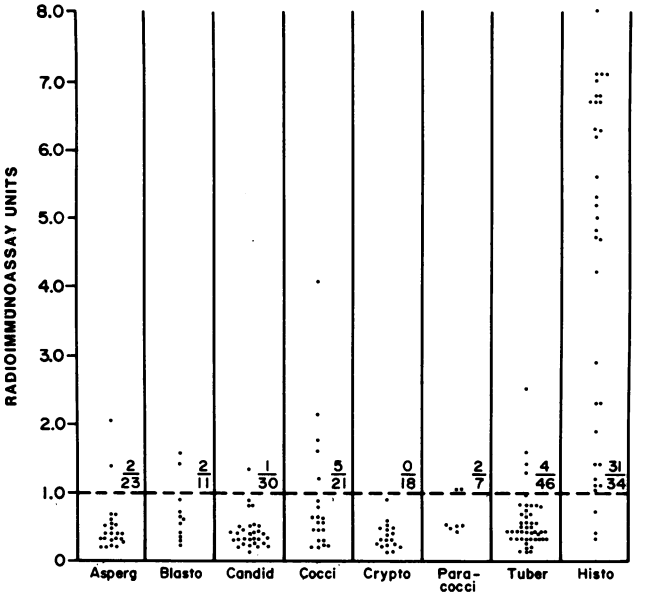

FIG. 3. IgM *Histoplasma* antibodies by RIA. Results exceeding one RIA unit are positive, indicated by the points above the horizontal dashed line.

ease from Los Angeles or from England suggests that the positive results in patients from Indiana and Illinois were caused by recent or past histoplasmosis, not by cross-reacting antigens. Otherwise, cross-reactions were infrequent, except with *Mycoplasma* infections, and were at relatively low levels compared with those of individuals with histoplasmosis.

Serum samples from 134 patients with clinically and laboratory-confirmed histoplasmosis (43) diagnosed during the Indianapolis outbreaks were tested by ID and CF against commercially available antigens of *B. dermatitidis*, *C. immitis*, *A. fumigatus*, and *H. capsulatum* to determine whether antibodies to *H. capsulatum* cross-reacted with

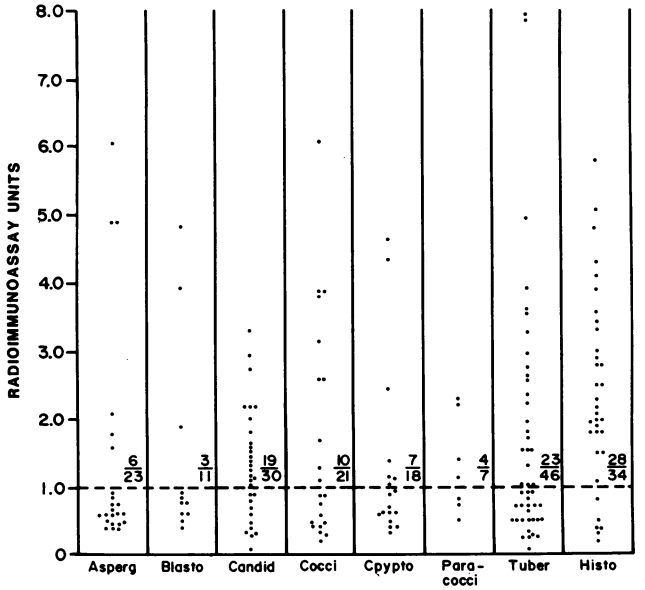

FIG. 4. IgG *Histoplasma* antibodies by RIA. Results exceeding one RIA unit are positive.

TABLE 1. Seropositivity among histoplasmosis patients and controls

Disease or control type	No. of patients positive/total (%) for the following tests:				
	ID	CF		RIA	
		Yeast	Mycelial	IgM	IgG
Histoplasmosis	22/34 (65)	28/34 (82)	7/34 (21)	31/34 (91)	28/34 (82)
Other fungal diseases	5/99 (5)	15/90 (17)	6/90 (7)	12/110 (11)	49/110 (45)
Tuberculosis	5/46 (11)	12/41 (29)	12/41 (29)	4/46 (9)	23/46 (50)
Other controls	0/123	12/105 (11)	9/105 (9)	17/123 (14)	28/123 (23)
Legionnaires disease	0/41[a]	9/31 (29)	4/31 (13)	3/41 (7)	12/41 (29)
England or Los Angeles	0/17	3/14 (21)	0/14	0/17	1/17
Illinois or Indiana	0/24	6/17 (35)	4/17 (24)	3/24 (12)	11/24 (46)
Mycoplasma pneumonia	0/25[a]	0/21	4/21 (19)	8/25 (32)	7/25 (28)
Indiana	0/10	0/7	0/7	2/10 (20)	2/10 (20)
Non-Indiana	0/15	0/14	4/14 (29)	6/15 (40)	5/15 (33)
Respiratory syncytial virus	0/10	1/8 (12)	0/8	0/10	0/10
Atypical pneumonia	0/10	0/8	0/8	0/10	2/10 (20)
Other pulmonary disease	0/37	2/37 (5)	1/37 (3)	6/37 (16)	7/37 (19)

[a] Fewer results are shown for ID and CF because of either insufficient serum or anticomplementary activity.

other common fungal pathogens. Of these, the following were judged positive by CF: 127 (40%) to *H. capsulatum*; 53 (40%) to *B. dermatitidis*; 20 (16%) to *C. immitis*; and 3 of 121 (2%) to *A. fumigatus*. By ID, 121 of 134 (90%) demonstrated H or M precipitins with histoplasmin, four (3%) demonstrated A antigen precipitins with blastomycin, and 2 of 121 (2%) demonstrated F antigen precipitins with coccidioidin, but none demonstrated precipitins with aspergillin. These specimens were not tested by RIA, since standardized RIAs for antibodies to these other fungal antigens have not been developed in our laboratory and are not commercially available. Presumably, even greater cross-reactivity with these different fungal antigens would have occurred in more sensitive assays such as the RIA.

To further assess the cause for cross-reactivity, a 1:5,000 dilution of serum from a patient with culture-proven cavitary histoplasmosis was incubated for 1 h at 37°C with a 1:10 dilution of antigenic extracts from *H. capsulatum*, *Candida albicans*, *C. immitis*, *B. dermatitidis*, *A. fumigatus*, and *Mycobacterium tuberculosis*. The *A. fumigatus*, *B. dermatitidis*, *C. immitis*, and *H. capsulatum* antigens were obtained from American MicroScan, the *C. albicans* whole-cell extract was obtained from Meridian Diagnostics, Cincinnati, Ohio, and the *M. tuberculosis* tuberculin antigen was obtained from the National Jewish Hospital Mycobacteriology Laboratory. The *Cryptococcus neoformans*, *Sporothrix schenckii*, and *Paracoccidioides brasiliensis* antigens were prepared in our laboratory by sonication of a 10% (vol/vol) concentration of yeast-phase organisms for 4 h by using a sonicator (Raytheon Co., Portsmouth, R.I.). Inhibition of IgG anti-*H. capsulatum* antibody activity was measured by RIA by comparison with a positive control containing the same serum specimen incubated with buffer alone. Each of these fungal extracts, as well as tuberculin, inhibited reactivity of the IgG anti-*Histoplasma* antibodies with histoplasmin (Fig. 5). The percent inhibition was calculated by the following formula: {[counts per minute (unabsorbed) − counts per minute (absorbed)]/counts per minute (unabsorbed)} × 100. In this experiment, the greatest cross-reactivity occurred between antigenic extracts of *H. capsulatum*, *P. brasiliensis*, *C. immitis*, and *B. dermatitidis*, with 77 to 81% inhibition. A lower degree of inhibition (35 to 56%) occurred with *A. fumigatus*, *C. albicans*, *S. schenckii*, *C. neoformans*, and *M. tuberculosis*.

To further assess cross-reactivity, specimens were selected from controls (with other fungal diseases or tuberculosis) which demonstrated high levels of antibodies to *H. capsulatum* as well as to the infecting fungus. These specimens were each incubated, first at 37°C for 1 h and then overnight at 4°C with a 1:10 dilution of their respective homologous extracts. After absorption, these sera were compared with the untreated positive control sera which had not been absorbed in an IgG RIA with tubes coated with either a 1:100 dilution of the homologous antigen (tuberculin was used at a 1:10 dilution) or histoplasmin. Absorption with the homologous antigen resulted in inhibition of IgG anti-

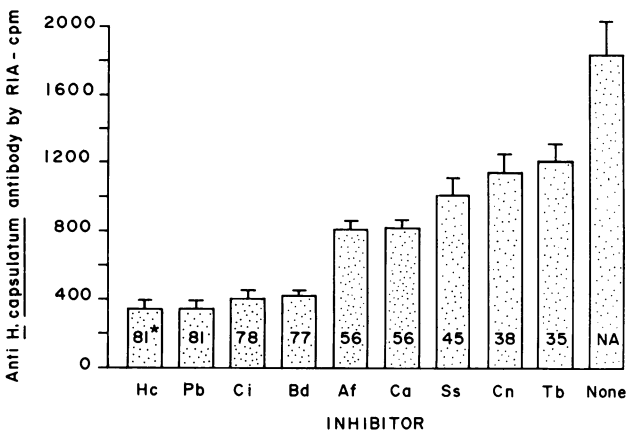

FIG. 5. Inhibition of IgG anti-*Histoplasma* antibodies measured by RIA. The experimental design is reviewed in Results. *, Percent inhibition of antibody activity after absorption of the positive control specimen {[counts per minute (unabsorbed) − counts per minute (absorbed)]/counts per minute (unabsorbed)} × 100 with the following antigens: Hc, *H. capsulatum*; Pb, *P. brasiliensis*; Ci, *C. immitis*; Bd, *B. dermatitidis*; Af, *A. fumigatus*; Ca, *C. albicans*; Ss, *S. schenckii*; Cn, *C. neoformans*; Tb, *M. tuberculosis*. The last column, labeled none, represents an unabsorbed positive control. NA, Not applicable.

TABLE 2. Effect of absorption with homologous antigen on homologous IgG antibodies and cross-reactive IgG anti-*H. capsulatum* antibodies by RIA

Infection type[a]	Radioactivity (mean cpm ± SD) before and after absorption for the following antibodies:					
	H. capsulatum			Homologous		
	Unabsorbed	Absorbed[b] (%)	*P*[c]	Unabsorbed	Absorbed[b] (%)	*P*[c]
Blastomycosis	1,865 ± 181	203 ± 20 (89)	<0.001	1,204 ± 42	204 ± 43 (83)	<0.001
Histoplasmosis	2,745 ± 124	470 ± 90 (83)	<0.001	NA[d]	NA	
Paracoccidioidomycosis	1,832 ± 120	565 ± 72 (69)	<0.001	1,630 ± 47	672 ± 102 (59)	<0.001
Aspergillosis	1,025 ± 100	328 ± 16 (68)	<0.001	668 ± 28	159 ± 13 (76)	<0.001
Coccidioidomycosis	1,315 ± 165	662 ± 103 (50)	<0.005	1,398 ± 207	200 ± 13 (86)	<0.001
Tuberculosis	1,085 ± 111	583 ± 95 (46)	<0.001	2,577 ± 181	660 ± 100 (74)	<0.001
Candidiasis	690 ± 42	440 ± 142 (36)	<0.05	2,530 ± 65	473 ± 20 (81)	<0.001
Cryptococcosis	915 ± 60	647 ± 97 (29)	<0.02	1,571 ± 52	449 ± 24 (71)	<0.001

[a] Infections are listed in decreasing order of percent inhibition of anti-*H. capsulatum* antibodies by absorption with the homologous extracts.
[b] Mean and standard deviation are based on triplicate tests. Percent inhibition is shown in parentheses. Percent inhibition = {[counts per minute (unabsorbed) − counts per minute (absorbed)]/counts per minute (unabsorbed)} × 100.
[c] *P* value is for a two-tailed Student *t* test comparing counts per minute in unabsorbed and absorbed specimens. See Materials and Methods for a description of the antigenic extracts used in the RIA to measure the homologous antibody response and for absorption.
[d] NA, Not applicable.

body activity to both the homologous antigen and to histoplasmin (Table 2), further establishing the presence of cross-reactive antigens in these different fungal and mycobacterial extracts.

DISCUSSION

We have demonstrated serologic cross-reactions to *Histoplasma* antigens among patients infected with other common fungal pathogens. Cross-reactivity was more frequent among patients with blastomycosis, coccidioidomycosis, paracoccidioidomycosis, and aspergillosis, but also occurred in patients with candidiasis and cryptococcosis. The false-positive rate would vary if higher or lower cutoff levels were used as criteria for positivity by CF or RIA. However, as more stringent criteria are used, the assay sensitivity drops proportionally. Irrespective of the exact cutoff chosen for positivity, these data support the occurrence of cross-reactivity among many fungal pathogens and mycobacteria. Cross-reactions to *H. capsulatum* antigens have been reported by others for aspergillosis (26), blastomycosis (11, 14, 21, 23, 26, 34–36, 38), candidiasis (37), cryptococcosis (21), coccidioidomycosis (4, 23, 26, 27, 31, 35), and paracoccidioidomycosis (37, 38), but few studies included sufficient controls to assess cross-reactivity accurately. Elevated levels of *Histoplasma* antibodies have also been reported for tuberculosis patients (21, 28, 30, 32). Additionally, sera from patients with fungal infections have reacted in tests for cellular and humoral responses to *Mycobacteria* (5, 12, 29). However, causes for elevated levels of fungal antibodies in patients with tuberculosis are more difficult to assess because these individuals frequently have concurrent fungal infections, particularly histoplasmosis and aspergillosis (10).

Concurrent or past histoplasmosis could have caused the tests to be positive for *Histoplasma* antibodies in some of the controls included in this study. We cannot disprove past or concurrent infection. However, the low rate (<5%) of seropositivity in normal controls residing in areas of the United States which are endemic for histoplasmosis (43, 44), and in patients with infections caused by viruses and bacteria other than mycobacteria, supports the hypothesis that *Histoplasma* antibodies in our patients with other fungal infections or tuberculosis usually were caused by cross-reacting antigens, not by concurrent or past histoplasmosis. Inhibition studies further support cross-reactivity rather than dual infection as a likely cause for positive serologic tests in these controls.

These inhibition studies established the presence of cross-reactive antigens among the different fungal pathogens and *M. tuberculosis*. Antibodies to *H. capsulatum* could be inhibited by absorption with crude antigenic extracts of *A. fumigatus*, *B. dermatitidis*, *C. albicans*, *C. immitis*, *C. neoformans*, *P. brasiliensis*, *S. schenckii*, and *M. tuberculosis*, as well as with *H. capsulatum* (Fig. 5). Furthermore, cross-reactive antibodies to *H. capsulatum* found in the serum of patients infected with fungal pathogens or *M. tuberculosis* were inhibited by absorption with the homologous antigenic extract (Table 2). These cross-absorption experiments were performed with a single serum specimen representative of each different fungal or mycobacterial control group included in the study, not with each of the 190 control specimens in these groups. Thus, we cannot conclude that positive results were caused by cross-reactivity rather than by concomitant or past histoplasmosis in every case. These inhibition studies did, however, establish the ability of infection with other fungi or mycobacteria to stimulate production of antibodies which cross-react with *H. capsulatum*. Furthermore, broad cross-reactivity compromises the ability of inhibition experiments to distinguish cross-reactivity from dual infection as a cause for seropositivity in individual cases.

Histoplasmin skin tests can result in false positivity by CF and ID in up to 15% of individuals with positive skin tests (22). Skin testing could have led to some of the false-positive results in patients included in this study. Because of this limitation of histoplasmin skin tests, they are primarily reserved for epidemiologic studies and investigations of outbreaks. Histoplasmin skin tests were not applied to patients at our medical center; however, such information was not available for specimens submitted from other investigators. Since histoplasmin skin tests are not commonly used and stimulate production of antibodies in a minority of patients, it seems unlikely that skin testing led to many of the positive serologic results seen in patients included in this study.

Common cell wall antigens have been identified in several fungi and presumably cause these cross-reactions (13, 16, 17, 25). Over 10 identical antigens have been found in *B.*

dermatitidis, *H. capsulatum*, and *C. immitis* (15, 18). Galactomannan is thought to be a major cause for cross-reactions and produces a precipitin band of identity with antigens from *H. capsulatum*, *P. brasiliensis*, and *B. dermatitidis* (2). A low-molecular-weight antigen extracted from *H. capsulatum* cross-reacts with *B. dermatitidis* and *C. immitis* (14). *H. capsulatum* H and M antigens are glycoproteins containing galactose, glucose, mannose, and hexosamine (1). Mannan is also found in cell walls of *Candida* (1, 18) and *Mycobacterium* spp. (9, 20), and glucan is found in cell walls of *B. dermatitidis*, *P. brasiliensis*, *Aspergillus* spp. (19), and *Mycobacterium* spp. (9). Galactan is found in *H. capsulatum*, *Mycobacterium* spp. (6, 9, 20), *Nocardia asteroides* (31), and *Corynebacteria* spp. (31). Mycobacterial cell walls also contain arabinogalactans, arabinomannans, mannans, glucans, and arabinose (6), all antigenic structures common to many fungi (39). Several of these cell wall antigens could cause serologic cross-reactions.

The problem of cross-reactivity compromises the usefulness of immunologic tests in fungal diseases (3, 7, 13, 24, 40–45). The lack of reliable serologic and skin tests has limited our understanding of the epidemiology, clinical features, and natural course of cryptococcosis (3, 24) and blastomycosis (7, 13, 24, 40). Cross-reactivity also complicates the interpretation of serologic tests of patients with histoplasmosis (22, 43, 44) and coccidioidomycosis (8). The significance of weakly positive results by CF (titers of 1:8 or 1:16) or RIA are particularly difficult to assess. Titers in this range may indicate active histoplasmosis (43), past histoplasmosis (43), or infections with organisms containing cross-reactive antigens. The immunodiffusion test is more specific if properly performed, by using appropriate control sera and standarized antigens, but it is less sensitive, thus compromising its usefulness as a screening test (42–44).

We previously expressed our opinion that serologic tests are valuable aids in the diagnosis of histoplasmosis (41–45), a position we continue to support. Although this study indicates that cross-reactions occur frequently in patients with other fungal diseases and probably in those with tuberculosis, several factors mitigate against abandoning these tests. First, cultural methods and special stains are insensitive methods for diagnosing self-limited infection, the most common manifestation of histoplasmosis (41, 43, 44). Serologic tests provide reasonable support for the diagnosis of self-limited histoplasmosis in patients with compatible illnesses and roentgenographic findings, thus eliminating the need for invasive and expensive diagnostic procedures. Second, positive serologic tests may provide the first clue to the diagnosis of histoplasmosis in patients with serious illnesses suggestive of disseminated disease or with chronic pulmonary disease compatible with cavitary histoplasmosis. *H. capsulatum* usually requires 3 to 4 weeks for isolation from positive cultures. These serologic findings may serve as the basis for other diagnostic studies to exclude histoplasmosis, for empiric therapy of severely ill patients while awaiting cultures, or as the sole laboratory basis for therapy in patients with negative cultures without established alternative diagnosis. Third, this study was designed to assess the potential for cross-reactivity by testing specimens collected from patients with other fungal diseases or tuberculosis, without attempting to control for the relative prevalence of these different infections. In an endemic area for histoplasmosis, a positive result in a serologic test for histoplasmosis would be more likely to represent a true-positive result; whereas in a nonendemic area, because of the lower relative prevalence of histoplasmosis, false-positive results in patients with other fungal disease or tuberculosis may occur more commonly. Epidemiologic, clinical, and other laboratory features help to distinguish the various fungal diseases. Furthermore, treatment decisions are usually similar for the different fungal diseases. Cross-reactivity with mycobacteria poses a more serious problem. Tuberculin skin tests and mycobacterial cultures are indicated in patients with positive serologic tests for histoplasmosis if the clinical findings are consistent with active tuberculosis or with progressive atypical mycobacterial disease.

Cross-reactivity was greatest with the RIA, as predicted by its greater sensitivity. Major advantages of the RIA over the standard assays are its ability to identify early cases not yet positive by CF or ID, and its ability to identify the immunoglobulin class of the antibody response. Our experience has revealed the RIA to be positive in 52% of sera obtained 3 weeks after exposure from patients identified during the Minnesota outbreak, compared with 0 judged positive by ID and only 6% judged positive by CF (unpublished data). Most of these early sera contained IgM antibodies to *H. capsulatum*, evidence for an acute infection. Our limited data have also shown that IgM antibodies clear earlier than IgG antibodies in patients with histoplasmosis (43, 45). Thus, despite the greater incidence of cross-reactivity by RIA, this technique has several features which support its continued development for the serodiagnosis of histoplasmosis.

More specific tests using purified antigens should overcome some of the problems with cross-reactivity and would be valuable aids in the diagnosis and management of systemic fungal infections. Until more specific tests become available, careful clinical judgment and additional laboratory examination should be used in assessing the significance of positive serologic tests in individuals with epidemiologic, clinical, and roentgenographic findings which are not highly suggestive of histoplasmosis, especially if the serologic tests are only weakly positive (22, 42, 43).

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