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Comparison and Diagnostic Value of the Coccidioidin Heat-Stable (HS and Tube Precipitin) Antigens in Immunodiffusion

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Coccidioides immitis **produces two unrelated heat-stable antigens which are diagnostically useful in immunodiffusion tests. One, the tube precipitin antigen, is valuable for specifically detecting antibody and diagnosing early primary cases of coccidioidomycosis. The other heat-stable antigen, designated HS, is the most useful coccidioidin antigen for specifically immunoidentifying *C. immitis* cultures. Both of the antigens were compared and evaluated for their usefulness in exoantigen and serologic immunodiffusion tests. Our studies indicated that the detection of tube precipitin antigen is of limited value for immunoidentifying *C. immitis* isolates because the antigen is common to certain gymnoascaceous saprophytes, such as *Arachniotus*, *Auxarthron*, and *Malbranchea* species, that form alternate arthroconidia. Antibodies to HS antigens are infrequently found in human sera from patients with coccidioidomycosis and are thus of little serodiagnostic value.**

In 1977, Standard and Kaufman (11) indicated that a serum specimen from a patient with coccidioidomycosis (lot 18311) reacted with mold-form *Coccidioides immitis* exoantigens to produce three specific immunodiffusion (ID) bands. Two of these bands consisted of antibodies reacting with heat-labile antigens, and one consisted of an antibody reacting with a heat-stable antigen. The antibody to one of the heat-labile antigens corresponded to the complement-fixing antibody and yielded a band identical to the ID complement fixation (IDCF) band (7). This band precipitated close to the antigen well. The antibody to the second heat-labile antigen produced a band designated IDHL which precipitated close to the IDCF band but closer to the serum well. The third band appeared to correspond to the reaction that was yielded in the tube precipitin (TP) test and was designated the ID tube precipitin (IDTP) band by Huppert et al. (7). Accordingly, the heat-stable exoantigen was referred to as the IDTP antigen. The antigen responsible for the IDTP reaction was heat stable at 60°C for 30 min and produced a band closer to the serum well than did the other two antigens. After the initial report of Standard and Kaufman, considerable work was done to further evaluate the specificity and reliability of the heat-stable antigen for identifying *C. immitis* (2, 3, 9). Because the heat-stable antigen and its antibody are diagnostically valuable for the immunoidentification of *C. immitis* and for the early serologic diagnosis of coccidioidomycosis, we decided to more closely examine the relationship between the reference heat-stable antigen used in exoantigen tests and that used in the IDTP tests for *C. immitis* immunoglobulin M antibody.

TABLE 1. Reactions of antigens for *C. immitis* and arthroconidium-forming fungi with different TP and exoantigen reference reagents

Fungal antigen extract	Reaction with *C. immitis* reference antigen (antiserum)			
	IDTP			IDHS exoantigen CIA-1-82 (HS-5-81) (CDC)
	CDN XV-B-73/67L (Mosher) (VA)	F/171 (UC)	CDN XV-B-73/67L (CH)	
C. immitis (10 strains)	+	+	+	+
Arachniotus reticulatus C-3	+	+	+	−
Arachniotus reticulatus C-72	+	+	+	−
Auxarthron zuffianum C-68	+	+	+	−
Auxarthron zuffianum C-69	+	+	+	−
Malbranchea filamentosa X-130	+	+	+	−

MATERIALS AND METHODS

Control antigens and antisera. Standardized coccidioidin reference antigens and *C. immitis* antisera used for serodiagnosing coccidioidomycosis by the ID test and for immunoidentifying *C. immitis* by exoantigen analysis were used. IDTP reference antigen CDN XV-B-73/67L and Mosher serum, an IDTP-positive control serum, were obtained from the Mycology Research Laboratory, Veterans Administra-

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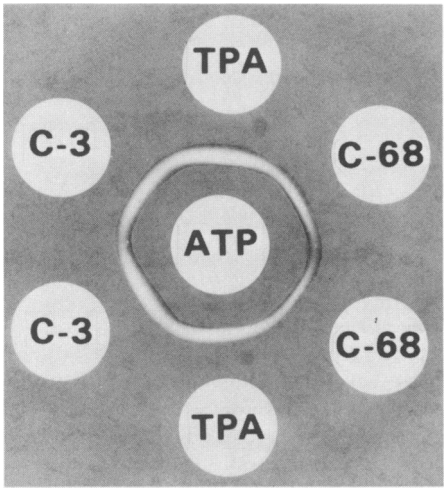

FIG. 1. Reactivity of *Arachniotus* and *Auxarthron* sp. exoantigens with *C. immitis* IDTP reference antiserum. TPA, UC IDTP antigen; ATP, UC IDTP antiserum; C-3, *A. reticulatus* antigen; C-68, *A. zuffianum* antigen.

tion Hospital (VA), San Antonio, Tex. The antigen was optimally diluted 1:6. IDTP antigen F/171 and a positive precipitin serum were provided by one of us (D.P.) in the Department of Medical Microbiology, University of California (UC), Davis. The antigen was used at its optimal dilution of 1:8. Goat antibody to *C. immitis* antigen 2 (1), the coccidioidin antigen considered responsible for TP activity, was provided by Rebecca Cox, San Antonio State Chest Hospital (CH), San Antonio, Tex. Heat-stable (HS) antigen CIA-1-82 diluted 1:3.5 and homologous HS-5-81 antiserum diluted 1.175 were used as exoantigen references and were from the Centers for Disease Control (CDC), Atlanta, Ga. The IDCF reference reagents included CDC *C. immitis* antiserum F-2 diluted 1:4 and coccidioidin antigen CIA-1-79 diluted 1:3. This antigen reacted with complement fixation test-positive sera to produce IDCF precipitates (5). IDCF and IDHS reference antisera were prepared by immunizing rabbits with multiple immunoelectrophoretic or ID precipitin arcs of IDHS and IDCF aggregates (4).

Test procedures. Published procedures were followed to test for exoantigens and antibodies by ID. Fungal cell-free antigens from agar slant cultures were tested for *C. immitis* exoantigens in the microimmunodiffusion test (10). Tests for IDCF, IDHS, and IDTP antibodies were carried out with the coccidioidomycosis microimmunodiffusion or macroimmunodiffusion test (8).

RESULTS

Preliminary studies were carried out to compare the reactions of three IDTP reference antibodies with the exoantigen IDHS antibody. Slant-culture extracts derived from 10 *C. immitis* strains, 2 *Arachniotus* sp. strains, 2 *Auxarthron* sp. strains, and 1 *Malbranchea* sp. strain were tested against IDTP and exoantigen reference reagents. The results (Table 1) revealed that the antigen which was identical to the IDTP reference antigens prepared by the VA, UC, and CH laboratories was produced not only by *C. immitis* but by the closely related saprophytic fungi studied. Figure 1 shows reactions between the UC *C. immitis* IDTP antiserum and culture filtrate antigens from *Arachniotus* and *Auxarthron* sp. strains. It was apparent that the *C. immitis* IDTP reference antigen was identical to the one produced by the saprophytes. In contrast, the antigen which was reactive with the CDC exoantigen reference antiserum was produced only by *C. immitis* (Table 1). This antigen, which, like the IDTP antigen, was resistant to heating at 60°C for 30 min, was designated the IDHS antigen. Our studies indicated that *C. immitis* isolates consistently produced both the IDTP and IDHS antigens.

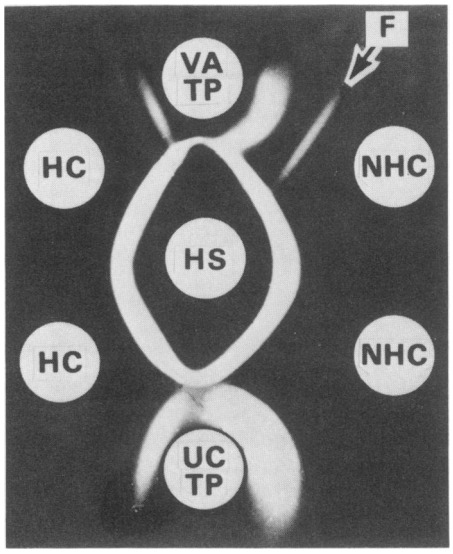

FIG. 2. Reactivity of IDTP and IDHS antisera with heated and nonheated coccidioidin. HS, CDC IDHS antiserum; VATP, VA IDTP antiserum; UCTP, UC IDTP antiserum; HC, heated coccidioidin; NHC, nonheated coccidioidin; F, IDCF band.

To further illustrate the differences between the IDTP and IDHS antigens and antibodies, we designed ID studies to compare the VA and UC IDTP antibodies with the CDC IDHS antibody. Each serum was tested against heated and nonheated coccidioidin (Fig. 2). The heated coccidioidin contained both the IDTP and IDHS antigens. The nonheated coccidioidin (VA lot XV-B-85F) contained both of the heat-stable antigens and the heat-labile IDF antigen. Our studies revealed that the VA IDTP antiserum contained antibodies to both the IDCF and IDTP antigens, whereas the UC IDTP antiserum contained antibody to only the IDTP antigen. The CDC IDHS antiserum contained only the IDHS antibody, which is unrelated to the IDTP antibody.

The reference reagents from UC, VA, and CDC were also compared in ID studies to further confirm the difference between the IDHS and IDTP reagents and to verify the

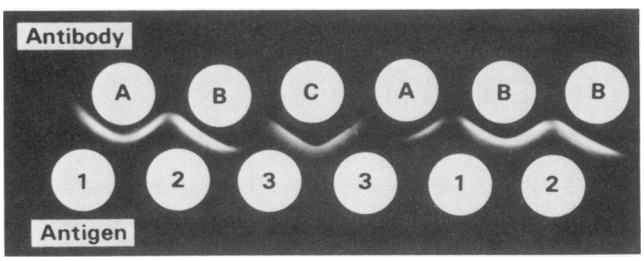

FIG. 3. Lack of identity of the IDTP and IDHS bands. The upper row of wells contains antisera to IDHS and IDTP antigens. The lower row of wells contains the IDHS and IDTP antigens. A, UC IDTP antiserum; B, VA IDTP antiserum; C, CDC IDHS antiserum; 1, UC IDTP antigen; 2, VA IDTP antigen; 3, CDC IDHS antigen.

TABLE 2. IDTP, IDHS, and IDCF reactions with sera from humans with early primary and advanced coccidioidomycosis

Serum[a]	Reaction with indicated reference precipitate			
	UC IDTP	VA IDTP	CDC IDHS	CDC IDCF
UC-OR	+	+	−	+
UC-NR	+	+	−	−
UC-EO142	+	+	−	ND[b]
UC-EO155	+	+	−	ND
UC-EO239	+	+	−	ND
UC-H6420	+	+	−	+
UC-H6421	+	+	−	−
UC-H6422	+	+	−	−
UC-H6432	+	+	−	+
UC-1	+	+	−	+
UC-2	+	+	+	+
UC-3	+	+	−	+
UC-4	+	+	−	+
UC-5	+	+	−	+
UC-6	+	+	−	+
VA-12	+	+	−	+
VA-Mosher	+	+	−	+
CDC 18311	+	+	+	+
CDC 84-047037	−	−	−	+
CDC 84-047132	+	+	−	+
CDC 84-057031	−	−	−	+
CDC 84-057215	+	+	−	+
CDC 84-057556	+	+	−	+
CDC 84-057781	−	−	−	+
CDC 85-003159	−	−	−	+
CDC 85-003469	+	+	−	+
CDC 85-003664	−	−	−	+

[a] UC and VA sera represent early primary sera. CDC sera represent advanced sera.
[b] ND, Not determined.

similarity between the UC and VA IDTP reagents. The results (Fig. 3) indicated that the IDTP reagents were identical and that they were distinguishable from the IDHS reagents.

Studies were carried out to determine how the specific *C. immitis* IDHS antigen would compare with the IDTP and IDCF antigens, which are commonly used for serodiagnosing early primary coccidioidomycosis and later stages of the disease, respectively. A total of 17 sera from early primary coccidioidomycosis patients and 10 sera from patients with more advanced coccidioidomycosis were tested for IgM antibodies with the UC and VA IDTP reagents and with the CDC ID tests for IDHS and IDCF antibodies. The IDTP reagents were positive with each of the 17 early sera studied, whereas only 1 (5.9%) of the early sera was positive for the IDHS precipitin (Table 2). A total of 14 of these 17 early sera were tested with the IDCF precipitin, and 11 were found to be positive. Of the 10 sera from patients with later stages of coccidioidomycosis, all were positive for the IDCF precipitin, whereas only 1 was positive for the IDHS precipitin.

DISCUSSION

Our studies indicated that the IDTP reference reagents prepared by the VA, UC, and CH laboratories were identical (Table 1 and Fig. 3). They also established that the IDHS reaction was different and distinct from those reactions which were classified as IDTP or IDCF reactions (Fig. 2 and 3). The IDTP antigen had been considered specific for *C. immitis*. Our study, however, revealed that the IDTP antigen, which is consistently produced by *C. immitis* cultures, is not unique to *C. immitis*. It is also produced by a variety of closely related arthroconidium-forming gymnoascaceous saprophytes, such as *Arachniotus*, *Auxarthron*, and *Malbranchea* species (Table 1 and Fig. 1). With the exception of *C. immitis*, the IDHS antigen was not produced by any of the arthroconidium-forming gymnoascaceous fungi studied. Obviously, the specificity of this antigen establishes it as a suitable target antigen for identifying *C. immitis*.

Both the IDTP and IDHS antigens are elaborated in the early stages of in vitro growth of *C. immitis*. However, it is interesting to note that whereas the IDTP antibody was invariably found in sera from patients with early acute primary coccidioidomycosis, the IDHS antibody was not (Table 2). In addition, the IDHS antibody was not a frequent component of sera from patients with the later stages of the disease. Concomitant use of the classical IDTP and IDCF tests permitted a specific diagnosis of coccidioidomycosis in all of the 27 sera studied, regardless of the stage of disease. The IDHS test, in contrast, was reactive with only two of these sera. The reason for the infrequent occurrence of IDHS antibody in human coccidioidomycosis sera is not clear. The antibody may be transiently present or present at such low concentrations that it cannot always be detected. Alternatively, the IDHS antigen appears to be the predominant antigen and may cause immune paralysis, or it may be overwhelmed in the host by the antigens that induce the early precipitin-type (IDTP) and the later CF-type (IDCF) antibody responses.

Coccidioidin is a heterogeneous preparation and contains multiple antigens (6). It is obvious that only standardized and defined reagents should be used in serodiagnostic and exoantigen coccidioidomycosis tests. If proper judgment is not exercised in the selection of reference ID precipitates for these tests, both false-positive and false-negative reactions will occur. The IDHS antigen is consistently produced by *C. immitis* in its early stages of growth. Because the IDHS antibody is specific for *C. immitis*, we recommend that it be used for the rapid immunoidentification of cultures of this pathogenic mold. The IDHS antigen, however, is unsuitable for serodiagnostic studies because the IDHS antibody is infrequently found in humans infected by *C. immitis*. The IDTP reference antigen is sensitive and specific for the detection of early *C. immitis* IgM antibody in human sera. We do not, however, recommend it for the immunoidentification of *C. immitis* cultures because the IDTP antigen, unlike the IDHS antigen, is produced by a variety of fungal saprophytes. To our knowledge, the production of IDTP antigen by saprophytic fungi does not compromise the value of serodiagnostic IDTP tests.

ACKNOWLEDGMENT

We dedicate this paper to the memory of the late Milton Huppert, a friend and colleague, who significantly contributed to the knowledge of coccidioidomycosis and its diagnosis.

LITERATURE CITED

1. **Cox, R. A., M. Huppert, P. Starr, and L. A. Britt.** 1984. Reactivity of alkali-soluble, water-soluble cell wall antigen of *Coccidioides immitis* with anti-*Coccidioides* immunoglobulin M precipitin antibody. Infect. Immun. **43**:502–507.
2. **DiSalvo, A. F., A. S. Sekhon, G. A. Land, and W. H. Fleming.** 1980. Evaluation of the exoantigen test for identification of *Histoplasma* species and *Coccidioides immitis* cultures. J. Clin. Microbiol. **11**:238–241.
3. **DiSalvo, A. F., A. A. Terreni, and A. K. Wooten.** 1981. Use of the exoantigen test to identify *Blastomyces dermatitidis*, *Coccidioides immitis* and *Histoplasma capsulatum* in mixed

cultures. Am. J. Clin. Pathol. **75**:825–826.
4. **Green, J. H., W. K. Harrell, S. B. Gray, J. E. Johnson, R. C. Bolin, H. Gross, and G. B. Malcomb.** 1976. H and M antigens of *Histoplasma capsulatum*: preparations of antisera and location of those antigens in yeast-phase cells. Infect. Immun. **14**:826–831.
5. **Huppert, M.** 1970. Standardization of immunological reagents, p. 243–252. Proceedings of the International Symposium on Mycoses. Scientific publication no. 205. Pan-American Health Organization, Washington, D.C.
6. **Huppert, M., J. P. Adler, E. H. Rice, and S. H. Sun.** 1979. Common antigens among systemic disease fungi analyzed by two-dimensional immunoelectrophoresis. Infect. Immun. **23**:479–485.
7. **Huppert, M., J. W. Bailey, and P. Chitjian.** 1967. Immunodiffusion as a substitute for complement fixation and tube precipitin tests in coccidioidomycosis, p. 221–225. *In* L. Ajello (ed.), Coccidioidomycosis. University of Arizona Press, Tucson.
8. **Huppert, M., and L. Kaufman.** 1972. Manual of standardized serodiagnostic procedures for systemic mycoses. Part 1. Agar immunodiffusion tests. Pan-American Health Organization, Washington, D.C.
9. **Huppert, M., S. H. Sun, and E. H. Rice.** 1978. Specificity of exoantigens for identifying cultures of *Coccidioides immitis*. J. Clin. Microbiol. **8**:346–348.
10. **Kaufman, L., and P. Standard.** 1978. Improved version of the exoantigen test for identification of *Coccidioides immitis* and *Histoplasma capsulatum* cultures. J. Clin. Microbiol. **8**:42–45.
11. **Standard, P. G., and L. Kaufman.** 1977. Immunological procedures for the rapid and specific identification of *Coccidioides immitis* cultures. J. Clin. Microbiol. **5**:149–153.