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SEROLOGY OF COCCIDIOIDOMYCOSIS

by

MILTON HUPPERT

Mycology Research Laboratory Veterans Administration Hospital, San Fernando, Calif., U.S.A.

For more than thirty years, coccidioidomycosis has been recognized as a primary respiratory infection resulting in symptoms which may simulate those of any other known respiratory disease. This infection is caused by the fungus, *Coccidioides immitis*, which appears to have a distribution restricted to the desert-like areas of the New World. Definitive diagnosis is accomplished best by isolation of the fungus or by demonstrating characteristic endosporulating spherules in diseased tissues. Frequently, this cannot be accomplished and one must rely on immunological tests as an aid for establishing a diagnosis. The work of Dr. C. E. SMITH and his colleagues (4) has established that several of these tests yield results useful not only for diagnosis but also for prognosis in this disease.

Recent evidence, however, has indicated that it is time to reinvestigate the question of the limited distribution of this fungus. We have reported that as many as 20 % of proven cultures of *C. immitis* have cultural characteristics which differ markedly from earlier descriptions (3). This raises the question, "How many times have we failed to recognize cultures like these as *C. immitis?*" In addition to this, we must note that Dr. GONZALEZ-OCHOA (1) has reported that coccidioidomycosis exists in at least two areas in Mexico where the climate and ecology is tropical rather than arid. Furthermore, persistent reports of coccidioidal-like disease in the Soviet Union have been related to me by Professor KASHKIN. These observations indicate that coccidioidomycosis may be present but not generally recognized in areas which have a climate other than desert-like and in countries outside of the New World. We now propose that these possibilities be investigated by immunological methods.

The clinical interpretations which can be derived from the established immunological tests are important to consider (Fig. 1.) Infection by *C. immitis* results in development of the delayed type of dermal hypersensitivity to coccidioidin. A positive skin test is

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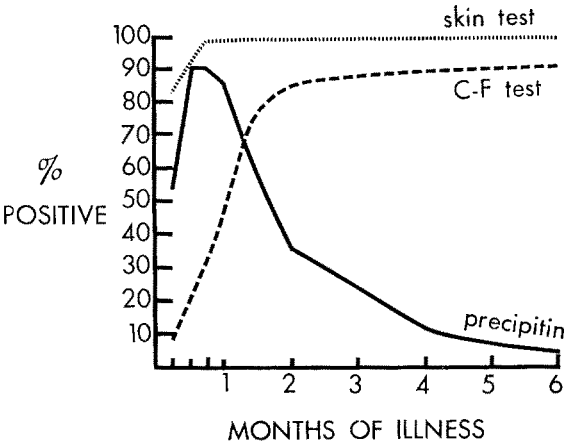

Fig. 1. Immunological reactions in symptomatic primary coccidioidomycosis relating time of appearance and duration to the frequency of positive reactions (Based on Dr. C. E. SMITH's data).

apparent usually during the first week of clinical disease and remains positive for long periods. This is a useful and practical test but a single positive result indicates only a past exposure to the fungus and not necessarily current active infection. For detection of recent disease, serological procedures are more valuable. The tube precipitin test usually becomes positive by the first week of clinical illness, but the frequency of positive results declines to less than 10 % by the fourth month. Therefore a positive tube precipitin test indicates early active disease. The complement fixation, or CF, test also becomes positive early but it tends to remain positive for longer periods than the tube precipitin test. Therefore, a single positive complement fixation test may indicate either early active disease or a residual positive from previous infection. It is important, however, that both serological tests be done, because the tube precipitin result may have reverted to negative by the time a specimen has been obtained or the complement fixation reaction may not yet be positive. Unfortunately, these two serological tests are not practical for studies in population groups. The complement fixation test requires too much time and special laboratory equipment, and the tube precipitin test presents some difficulty in obtaining reproducible readings and high sensitivity. For these reasons we have evaluated an agar-gel immunodiffusion method as a substitute for complement fixation, and latex particle agglutination as a substitute for the tube precipitin test (2). The immunodiffusion (ID) and latex particle agglutination tests were chosen because earlier studies had shown that they have a high level of sensitivity, that they require only minor equipment that is adaptable to use in the field, that many specimens can be tested in a few minutes, and that results are obtained in four minutes with the latex particle agglu-

TABLE I

Comparison of tube precipitin (TP) and latex particle agglutination (LPA) tests for diagnosis (DX) of coccidioidomycosis

Test	Results				
	A	B	C	D	% of total DX positives (D/B+D) × 100
	Test o DX o	Test o DX +	Test + DX o	Test + DX +	
TP	170	115	2	18	13.5 (8—21) [1]
LPA	167	42	10	101	70.6 (62—78)

[1]) Figures in parenthesis represent the range which will encompass the true value with a confidence level of 95 %.

TABLE II

Comparison of complement fixation (CF) and immunodiffusion (ID) tests for diagnosis (DX) of coccidioidomycosis

Test	Results				
	A	B	C	D	% of total DX positives (D/B+D) × 100
	Test o DX o	Test o DX +	Test + DX o	Test + DX +	
CF	154	23	3	115	83.4 (76—89) [1]
ID	176	27	1	118	81.4 (74—88)

[1]) Figures in parenthesis represent the range which will encompass the true value with a confidence level of 95 %.

TABLE III

Comparison of combined LPA-ID results with combined TP-CF results for diagnosis (DX) of coccidioidomycosis

Test	Results				
	A	B	C	D	% of total DX positives (D/B+D) × 100
	Tests o DX o	Tests o DX +	Tests + DX o	Tests + DX +	
LPA-ID	165	10	11	133	93.0 (87—97) [1]
TP-CF	149	21	5	115	84.6 (77—90)

[1]) Figures in parenthesis represent the range which will encompass the true value with a confidence level of 95 %.

tination test and usually in one day with the immunodiffusion test. The validity of this approach is demonstrated in Tables I-III in which the test results are compared with the clinical diagnosis of whether the patient had or did not have coccidioidomycosis. Table I shows that the latex particle agglutination (or LPA) test was positive with 70.6 % of the specimens from patients with coccidioidomycosis, and this compares to only 13.5 % for the tube precipitin (or TP) test with the same specimens. Furthermore, only 10 of the total 320 specimens were LPA positive when the diagnosis was negative, indicating a low level (3 %) of false positive results. The very high level of sensitivity achieved with the LPA test more than compensates for this low level of false positive reactions. Table II compares the results obtained with the complement fixation test and the immunodiffusion test. In this case the results with the two tests are almost identical, with positive reactions in more than 80 % of the cases. Table III illustrates that a combination of the LPA and ID tests is probably better than a combination of TP and CF tests for detecting antibodies to *C. immitis*, the LPA-ID combination yielded positive results in more than 90 % of the cases.

At this point we are confident that the techniques selected are appropriate for determining whether or not coccidioidomycosis occurs in previously unrecognized areas. A skin testing survey would reveal the prevalence of past exposure to *C. immitis* within a population group. The latex particle agglutination and the immunodiffusion tests could be used to rapidly and accurately screen large numbers of specimens to detect humoral antibodies as an indication of active disease.

One major problem remains. Since strains of *C. immitis* may appear in many cultural varieties and since many of these have not been recognized in the past, a similar variation in antigenic types may exist among the more recently recognized strains of this fungus. If this is true, then antigens prepared from strains recovered from one area might be immunologically different from strains occurring in another area. Under these conditions, it might not be appropriate to employ antigens prepared from cultures indigenous to the Western Hemisphere for testing among population groups in other parts of the world. For example, before our current standard American antigens are used in any other country, we must demonstrate that these coccidioidins contain the same antigenic composition as coccidioidins prepared from strains found in that country. We have used the immunodiffusion method to compare the antigenic composition of the various strains of *C. immitis* in our collection, and even though all our strains are from the western hemisphere, this problem of immunologic types still exists. As shown in the diagram in fig. 2, we designed an immunodiffusion pattern to detect the presence of four specific antigen-antibody systems in coccidioidomycosis. The two center wells, labelled As, contain antiserum, and the four corner wells contain our reference antigens made from

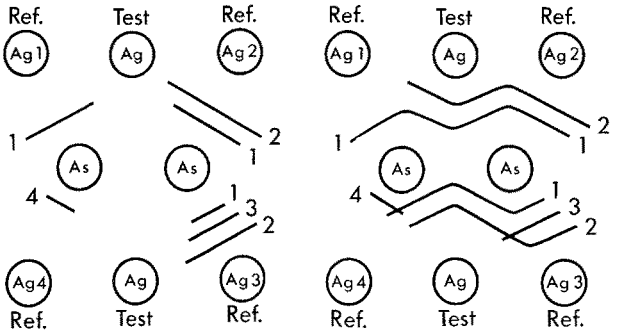

Fig. 2. Diagram of immunodiffusion test pattern illustrating the four identifiable reference antigen (Ref. Ag) and antiserum (As) reactions.

Fig. 3. Diagram of a typical reaction of a test antigen with the reference systems. In this case the test antigen solution contains antigens 1 and 2.

American strains of *C. immitis*. At present we do not have antigen preparations which contain only a single molecular species, so some of the specific lines of precipitation are repeated in the four reference antigen systems. Among the precipitation lines shown there are four which are not-identical with any other system, and these are numbered as indicated. The two wells labelled "Test Ag" are used for the coccidioidin prepared from a strain to be analyzed. Fig. 3 is a diagram that illustrates how this pattern of reactions can be inter-

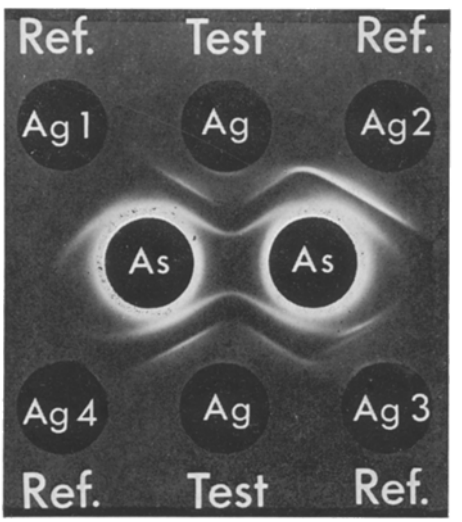

Fig. 4. Photograph of immunodiffusion test diagrammed in Fig. 3.

preted. In this instance the test antigen solution contains antigens identified as number 1 and number 2 by the fusion of precipitation lines with those formed by the reference antigen systems. Fig. 4 is a photograph of the immunodiffusion plate which was diagrammed in the previous figure. This photograph was taken after two days of incubation. Precipitation line 4, which was apparent after one day of incubation, has been masked by the extension of one of the other precipitation lines.

With this method we have begun the analysis of many strains in our collection. Table IV presents some representative results to

TABLE IV

Analysis of antigens from "atypical" strains of *C. immitis*

Culture No.	Antigens 1	2	3	4
321	+	+	+	+
109	+	+	+	o
173	+	+	+	o
83	o	+	+	+
51	o	+	+	+
24	o	+	+	+
88	o	+	+	+
333	o	+	+	o
97	o	o	o	o

illustrate the existence of immunological types among strains of *C. immitis*. It can be seen that some strains produce all four antigens while others do not. There is even one strain which does not produce any antigen reactive with the four reference systems which we have been using, and for this one a new reference system must be developed. We have identified Antigen 2 as the specific antigen involved in our standard complement fixation and immunodiffusion tests. Antigen 1 is the one involved in the standard tube precipitation and latex particle agglutination tests. Since many strains of *C. immitis* lack Antigen 1, the importance of employing native strains for the preparation of coccidioidins to be used in any specific area is emphasized.

Summary

We believe there is strong evidence to support a continuing search for coccidioidomycosis in new areas, in the Old World as well as in the New World, and in places with a climate and ecology different from the semi-arid conditions of the known endemic areas. Such an investigation would be justified in any population group where there is a high incidence of respiratory disease of unknown etiology. Very satisfactory and practical immunological techniques are available, but the present evidence indicates that the antigens used in these tests should be prepared from

strains of *C. immitis* recovered from the area to be investigated. Obviously this cannot be done at present, so such a program should be preceeded by an extensive survey of soil samples in the area in order to recover any existing native strains of *C. immitis*. Whereas this would be the ideal situation, one could consider initiating the proposed study with coccidioidins prepared from selected strains of this fungus, incorporating as complete a spectrum of known antigens as is possible with our present knowledge, and keeping in mind that even this may not be adequate. We would welcome the opportunity to assist any investigator preparing to undertake a survey for coccidioidomycosis in his country.

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