Agar-Gel Precipitin-Inhibition Test for Coccidioidomycosis

I. Preliminary Evaluation of the Complement-Fixation and Agar-Gel Precipitin Tests in the Serodiagnosis of Human Coccidioidomycosis

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The agar-gel precipitin-inhibition serological test for coccidioidomycosis was a more sensitive indicator of *Coccidioides immitis* antibodies than the tube precipitin, the agar-gel immunodiffusion, in the complement-fixation tests in assaying monkey sera, whether these sera were from prechallenge-vaccinated or postchallenged animals. When applying this technique to the assay of human sera, an analogous finding generally persisted. However, some human sera were positive by the complement-fixation test and negative by the agar-gel precipitin-inhibition test. These sera were diffused in agar-gel against various coccidioidin complement-fixation, tube precipitin, and agar-gel precipitin-inhibition test antigens with essentially negative results.

In previous reports (2, 3), the agar-gel precipitin-inhibition (AGPI) and complement-fixation (CF) tests for coccidioidomycosis were compared for their ability to assay *Coccidioides immitis* antibodies in monkey and dog sera. In addition, the tube precipitin (TP) and the agar-gel immunodiffusion (AGID) tests (1) were compared with the above serological tests in one set of experiments (2). The results indicate that the AGPI procedure was more sensitive than the CF, TP, or AGID tests in determining the *C. immitis* antibody content of these monkey sera, whether they were from prechallenged-vaccinated or post-challenged animals.

However, sufficient human serum specimens were not available at that time to perform an analogous assay. During the past year, a significant number of human coccidioidomycosis sera were obtained through the courtesy of Evelyn Wallraff, Veterans Administration Hospital, Tucson, Ariz., H. Gilbert Crecelius, Arizona State Department of Health, and John Bennett, National Institutes of Health Clinical Center. Comparative assays on these human sera are reported here.

MATERIALS AND METHODS

An agar-gel medium previously described by Ray and Kadull (4), a coccidioidin antigen prepared and supplied by John Converse, and a coccidioidomycosis antiserum, human or animal, of high CF titer (1:256 or greater) were employed in the AGPI test. Standardization of the coccidioidomycosis AGPI test antigen-

antibody system is attained by a box titration in agar-gel of serial dilutions of each reactant. This determines the minimal reacting dilution of test antigen and antibody that shows a visible precipitate in the agar-gel plates within 48 hr. Any inhibition of this test reaction by an unknown serum indicates the antibody titer of that serum. To obtain a visible reaction equidistant between the agar-gel wells, it is necessary to prediffuse the test antibody minimal reacting dilution, located in the center wells, 1 hr at 23 to 27 C (3).

The CF titers in this report were attained by the source laboratory technique or by the Kolmer 100% fixation technique as used in our Fort Detrick Serology Laboratory. The latter technique utilized the recommended Communicable Disease Center CF antigen and human control antiserum.

The direct double diffusion tests of the CF positive-AGPI negative sera were performed in agar-gel plates having six wells equidistant from a center well (Fig. 1-3). The center wells contained the investigated sera, and the peripheral wells were charged with six different antigens. Each reagent was placed in its respective well every 24 hr for 5 days. After the initial 5-day serial recharging with the reagents, the wells were recharged 24 hr before the plate reactions were to be photographed. The incubation time was extended for 21 days at 23 to 27 C, and the reagents were not diluted for this series of tests. This treatment varied from the standard initial charging of reagents and observation periods of 48 to 72 hr without further recharging in an effort to negate differences in concentration of the antigen or antibody content in these reagents.

RESULTS AND DISCUSSION

The AGPI procedure detected titers in prechallenged-vaccinated and postchallenged mon-

TABLE 1. Comparison of serological results in monkey's response to cutaneous and pulmonary coccidioidomycosis

Immunization	Monkey	Prechallenge reciprocal titer (6 months postvaccination)			15 days postchallenge (Silveira ^a) reciprocal titer	
		Skin test	CF	AGPI	CF	AGPI
Unvaccinated (controls)	T-40	—	—	—	—	—
	T-34	—	—	—	—	—
	T-74	—	—	—	—	—
	TR-74	—	—	—	—	—
	T-66	—	—	—	—	—
Viable vaccine (Silveira ^a spores)						
	Dose 10					
	T-78	+	32	64	64	64
	S-22	+	—	8	8	64
	T-67	+	—	8	16	32
	T-44	+	—	4	8	32
	Dose 100					
	T-38	+	16	32	32	32
	T-30	+	—	4	8	16
	T-77	+	—	2	—	8
	T-57	+	—	4	8	16
	Dose 1,000					
	T-63	+	—	32	8	32
	T-21	+	16	64	16	64
	T-17	+	—	16	—	32
	T-43	+	256	512	256	512
Nonviable vaccine (Formalin-killed Cash strain)						
	S-38	± ^b	—	—	—	8
	S-13	±	—	—	—	4
	TR-76	±	—	—	—	2
	TR-75	±	—	—	—	32

^a Refers to a strain of *Coccidioides immitis*.^b Questionable results (erythema but no induration).

TABLE 2. Comparison of postchallenge serological reactions in immunized and nonimmunized monkeys

Immunizing agent	Monkey	Disease status ^a	Tube precipitin			AGID			Reciprocal titer AGPI			Complement fixation					
												Huppert			Ray		
			2 wk	8 wk	12 wk	2 wk	8 wk	12 wk	2 wk	8 wk	12 wk	2 wk	8 wk	12 wk	2 wk	8 wk	12 wk
Intact spherule	K-21	3+	0	0	0	±	+	+	32	512	1,024	8	0	2	20	0	80
	K-43	2+	0	NS ^b	+	0	+	+	4	128	1,024	0	8	8	NS	160	640
	K-11	1+	0	0	+	0	+	+	4	128	256	0	8	4	NS	80	160
	K-20	2+	0	0	0	0	+	+	0	128	1,024	0	0	0	0	0	0
	P-17	1+	0	0	0	0	+	+	0	32	256	0	AC ^c	128	0	80	640
Combined cell fraction	S-49	2+	0	0	+	0	0	+	0	8	4	0	0	0	0	0	0
	K-45	3+	0	0	+	0	+	+	0	512	1,024	0	8	512	AC	80	1280
	P-12	2+	0	0	+	0	+	+	0	256	1,024	0	16	256	0	640	640
	P-14	1+	0	0	+	0	+	+	0	128	1,024	0	4	32	0	320	320
	P-15	2+	0	0	0	0	0	+	0	16	8	0	0	2	0	10	10

^a Relative extent of infections: 1+, mild; 2+, moderate; 3+, severe.^b No sample.^c Anticomplementary.

TABLE 3. *Comparative human coccidioidomycosis serum titrations*

Serum	Reciprocal titers		
	Source CF	FDSL ^a CF	AGPI
Gep	Negative	Negative	2
Hod	8	10	32
Pav	Negative	Negative	Negative
Suk			
7-14-65	Negative	40	32
7-17-65	Negative	40	32
7-22-65	Negative	20	16
C.L.			
9-22-64		40	256
10-13-64	16	40	128
11-24-64		20	64
12-1-64	32	20	64
2-23-65	16	20	32
R. C.			
7-14-64		80	256
8-18-64		80	256
9-15-64		80	128
10-6-64		80	128
11-24-64		40	64
8-24-65		40	64
1-4-66		20	16
2-8-66		20	32
CDC ^b lot 6 human control serum	64	80	128

^a Fort Detrick Serology Laboratory.^b Communicable Disease Center.

key sera when the CF tests were negative and permitted earlier titer evaluation; these titers generally persisted longer than those assayed by the CF technique (Table 1).

When the AGPI test results were compared with those of the CF, TP, and AGID tests in an interlaboratory investigation of sera from immunized and nonimmunized postchallenge monkeys, the AGPI test detected earlier titers in two of five sera in the spherule vaccine group and in two of five sera in the combined cell fraction group (Table 2).

In the combined cell fraction vaccine group, only the TP, AGID, and AGPI tests detected antibody response in the S-49 monkey serum; the CF tests were negative. Antibodies to *C. immitis* in the K-20 serum of the intact spherule vaccine group were detected only by the AGID and AGPI tests. It is also interesting to note in this vaccine

group series that the Huppert CF test results in the 2-, 8-, and 12-week serum samples were either equivalent in titer or decreased; the AGPI test results, however, indicated a decided increase in titer over these same time periods. Although the reason for this is unknown, the possibility exists that the two laboratories may have measured different antigen-antibody reactions.

Human sera were also compared by the AGPI and CF techniques. The first samples titrated were those obtained from E. Wallraff and J. Bennett, and the titers were compared by the CF and AGPI test procedures (Table 3). The second group of human sera was obtained from H. Gilbert Crecelius, and the titers were also compared by these test procedures (Table 4).

Three sera (17737, 17979, and 18961) having positive LBCF titers (1:8, 1:4, and 1:32, respectively) were negative by the AGPI test (Table 4). These findings are at variance with those obtained with monkey sera and could be caused by either differences in sera or differences in the antigens that assay their antibody content. Serum 17737 came from a patient with a positive skin test,

TABLE 4. *Comparative human coccidioidomycosis sera titrations*

Serum	Reciprocal titer ^a		
	Source LBCF	FDSL CF	AGPI
17202	16	Negative	32
17204	64	10	128
17206	64	80	128
17338	64	40	128
17729	32	10	128
17730	64	40	128
17731	128	40	256
17737	8	Negative	Negative
17843	8	Negative	32
17848	8	Negative	16
17973	16	10	16
17979	4	Negative ^b	Negative
18113	8	5	16
18184	16	10	16
18185	8	5	32
18799	16	10	64
18961	32	Negative ^b	Negative
CDC ^c lot 6 human control serum	64	80	128

^a LBCF = Laboratory Branch Complement Fixation test as proposed by the Laboratory Branch of the National Communicable Disease Center, Atlanta, Ga.; FDSL = Fort Detrick Serology Laboratory.

^b Anticomplementary serum.

^c Communicable Disease Center.

positive X ray, and positive culture of *C. immitis* from bronchial washings; serum 18961 came from a patient with a positive skin test, X ray, and culture from animal inoculation; no case history was available on serum 17979.

Each of these sera was diffused in agar-gel against six antigen preparations. M. Huppert supplied four antigens: the XV-B-60F, UFR coccidioidomycosis CF antigen, the XV-B-60L coccidioidomycosis precipitin antigen, the XV-B-60L IDTP antigen, and the XV-B-60F, UFR IDCF antigen. In addition, lot 8 CF antigen was obtained from the Communicable Disease Center, and lot 3 coccidioidin was obtained from John Converse. These six antigens were employed in the agar-gel double diffusion of the above sera as well as of a positive control serum, 17730 from H. G. Crecelius' laboratory, and lot 6 Communicable Disease Center human positive CF control serum. Each reagent was placed in its respective well every 24 hr for 5 days. After the initial 5-day serial recharging with the reagents in their wells, the wells were recharged 24 hr before the plate reactions were to be photographed. This treatment tends to negate differences in concentration of the antigen or antibody content in these reagents.

Figure 1 depicts the serial diffusion of the reagents and shows that no activity was detected from sera 17979 and 17737 (both negative by the AGPI technique and positive by the LBCF method).

Figure 2 shows that the positive control serum 17730 and lot 6 Communicable Disease Center CF human serum control were both positive, to varying degrees, with the diffused antigens.

After continuing this procedure for 18 and 21 days (Fig. 3), a weak antigen-antibody reaction appeared between the two sera, 17737 and 17979, and lot 3 AGPI and XV-B-60F (UFR coccidioidomycosis CF) antigens. To determine the significance of this measured antigen-antibody reaction would require a coccidioidomycotic test antigen-antibody evaluation.

This present experiment, however, shows that these antigens in the standard test concentrations would not give an antibody titer of coccidioidomycosis with these negative sera, either by inhibition of the antigen in the AGPI test or by binding of complement in the CF test. Similarly, AGID antigens would not have detected the presence of coccidioidomycosis antibodies in these sera, because a larger total concentration was employed in both reagent wells and a longer observation period was used. However, certain CF test antigens did measure an apparently different antibody content in suspect coccidioidomycotic sera as a result of differences in antigenic content. This observation requires further investigation.

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Tables 1 and 2 are taken from *Coccidioidomycosis: Pertinent Findings and Developments*, Arizona State Department of Health, University of Arizona Press, 1967.

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