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Use of a Recombinant *Coccidioides immitis* Complement Fixation Antigen-Chitinase in Conventional Serological Assays

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The coccidioidal complement fixation (CF) antigen has been cloned previously, and the fusion protein has been expressed in *Escherichia coli*. The recombinant CF (rCF) antigen was affinity purified by adsorption-desorption to chitin, and its reactivity was studied by using sera containing coccidioidal antibodies. The affinity-purified rCF antigen formed a line of identity with an immunodiffusion (ID) CF reference antigen (coccidioidin) derived from mycelial-phase *Coccidioides immitis* and was reactive with human, canine, and equine sera containing coccidioidal antibody. The affinity-purified rCF antigen yielded no detectable reaction with *Blastomyces* or *Histoplasma* antiserum by ID. The affinity-purified rCF antigen fixed complement with positive human sera and, even when used at lower concentrations, yielded titers comparable to those obtained with the coccidioidin. The reactivity of the affinity-purified rCF antigen was further evaluated by enzyme immunoassay, in which it manifested good sensitivity (96.9%) and specificity (100%) when evaluated with 43 human patients' sera. Thus, the affinity-purified rCF antigen has yielded reactions comparable to those of crude coccidioidal antigens in conventional CF, IDCF, and enzyme immunoassay.

Diagnosis of coccidioidomycosis is often made on the basis of serological assays employing crude antigens prepared from filtrates or lysates of mycelial or spherule-endospore phases of *Coccidioides immitis* (25). Currently used qualitative tests include immunodiffusion (ID) for the detection of immunoglobulin M (IgM) (IDTP) or IgG (IDCF) and enzyme immunoassay (EIA) for the detection of these two major antibody responses (4, 19). Quantitation of antibody, primarily IgG, is carried out by complement fixation (CF) or quantitative ID, and the titer is related to the extent of coccidioidal disease (8, 22). The EIA, while useful for qualitative detection of antibody, has not yet provided a satisfactory substitute for the quantitative results of the more tedious and complicated CF test (14). In part, this may be due to the unavailability of appropriate purified antigen(s) for EIA determination. It is possible that such a purified antigen with potential for detection of coccidioidal IgG could be produced by recombinant molecular technology.

We have identified the antigen, a chitinase, that participates in CF and the corresponding IDCF assay (10) and have isolated a cDNA clone from a 24-h-old developing-spherule cDNA library that encodes the CF chitinase protein (27). We have subsequently expressed the recombinant protein in *Escherichia coli* and have shown it to have antigenic and enzymatic activities similar to those of the native *C. immitis* CF chitinase protein (27). Yang et al. (24) have also isolated a cDNA clone that exhibits chitinase activity and is reactive in Western blots (immunoblots) with CF antibodies. We here present data comparing the recombinant CF protein with the crude coccidioidal antigen used in conventional serological tests.

MATERIALS AND METHODS

Microbial strains, plasmids, and medium. *E. coli* XL1-Blue (Stratagene, La Jolla, Calif.) was used as the host for all plasmids. Plasmid pCTS 4-2A contains the entire message encoding the *C. immitis* CF antigen inserted into the plasmid pBluescript (Stratagene). Plasmid pCTS 4-2A was isolated as previously described (27) from a developing-spherule cDNA library. This cDNA library was prepared from mRNA isolated from 24-h-old developing *C. immitis* Silveira (ATCC 28868) spherules. Briefly, the clone was isolated from the cDNA library by using a ^{32}P-labeled, PCR-generated, chitinase-specific amplimer probe. This probe was generated from *C. immitis* genomic DNA by using primers synthesized from the combined CF-chitinase N-terminus amino acid sequence (11) and from the presumed bacterial and fungal internal active-site consensus amino acid sequence (1, 6, 12, 23). The pCTS 4-2A insert DNA sequence is deposited in GenBank under accession no. U60807 (27). *E. coli* cells were cultured in Luria-Bertani medium (1% tryptone, 0.5% yeast extract, 1% NaCl [pH 7.0]) at 37°C as indicated below.

Preparation of *E. coli* extracts. Extracts of *E. coli* cells containing the plasmid pCTS 4-2A or pBluescript were prepared by an osmotic shock procedure as previously described (16, 27). Briefly, *E. coli* cells were grown in Luria-Bertani medium containing 50 μg of ampicillin per ml at 37°C with shaking for 2 h. Isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to the cultures to a final concentration of 1 mM to induce production of the recombinant fusion protein, and the incubation was continued overnight. The cells were harvested by centrifugation at 10,000 × *g* for 10 min at 4°C, and the supernatant was discarded. Cell pellets were suspended to a concentration of 5 optical density units/ml (optical density measured at 550 nm) in ice-cold 20% sucrose–20 mM Tris-HCl (pH 8.0)–2.5 mM EDTA, chilled on ice for 10 min, and centrifuged as described above. The absorbance was measured with a lambda 3A UV–visible-spectrum spectophotometer (Perkin-Elmer, Norwalk, Conn.). The supernatant was removed and discarded, and the cell pellet was resuspended in ice-cold 20 mM Tris-HCl (pH 8.0)–2.5 mM EDTA and placed on ice for 10 min to lyse the bacteria. The cell debris was pelleted by centrifugation at 13,000 × *g* for 30 min at 4°C. The supernatant was collected, sterilized by passage through a 0.2-μm-pore-size filter (Nalge, Rochester, N.Y.), and concentrated approximately 100-fold with a stirred pressure cell fitted with a 10,000-molecular-weight-cutoff membrane (Amicon, Danvers, Mass.).

Purification. Purification of the recombinant CF (rCF) antigen from the *E. coli* lysates was carried out by the affinity adsorption-desorption procedure previously described for the purification of a chitinase from *Serratia marcescens* (2) and used to isolate the coccidioidal CF-chitinase (10). This procedure is based on the binding of the enzyme to chitin and recovery of the enzyme after digestion of the substrate. Regenerated chitin (reacetylated chitosan), prepared as previously described (2, 10), was used as the substrate. Chitin was added to the concentrated osmotic *E. coli* extract to provide 5 mg (dry weight) of chitin per ml of extract and incubated overnight at 4°C to permit adsorption of the enzyme to the chitin substrate. Following washing by centrifugation, the chitin-chitinase complex was resuspended in potassium phosphate buffer, pH 6.3, and incubated overnight at 37°C. The soluble chitinase, released after digestion, was recovered in the supernatant after centrifugation to sediment the remaining chitin and was sterilized by passage through a 0.2-μm-pore-size filter (Gelman, Ann Arbor, Mich.).

Protein. Protein concentration was estimated by the method of Lowry et al. (17) with bovine serum albumin as the standard.

Gel electrophoresis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed by using the discontinuous buffer system of

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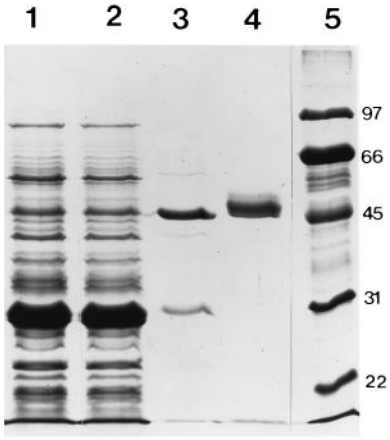

FIG. 1. Reduced SDS–12% PAGE gel. Lane 1, osmotic lysate of *E. coli* containing the nonrecombinant plasmid pBluescript (20 μg of protein); lane 2, osmotic lysate of *E. coli* containing the recombinant plasmid pCTS 4-2A (20 μg of protein); lane 3, rCF antigen affinity purified from *E. coli* containing the recombinant plasmid pCTS 4-2A (20 μg of protein); lane 4, native coccidioidal CF/chitinase affinity-purified from 48-h spherule-endospore-phase culture filtrate (4 μg of protein); lane 5, molecular mass markers (numbers are in kilodaltons).

Laemmli (15). Prior to loading, the samples were reduced by boiling for 4 min in the presence of mercaptoethanol. Proteins in their native state were similarly electrophoresed except that all buffers were free of SDS and mercaptoethanol and the samples were not heated. Gels were stained with Coomassie blue (5). The relative mobility markers (Bio-Rad, Hercules, Calif.) ranged from 97 to 14 kDa.

Sera. Human, canine, and equine samples were obtained from the University of California, Davis, Coccidioidomycosis Serology Laboratory, where they had been tested for coccidioidal antibodies. *Histoplasma* and *Blastomyces* immunodiffusion antisera (Meridian Diagnostics, Cincinnati, Ohio) were used to evaluate cross-reactivity.

Antigens. Coccidioidal antigens F171 and CF pool 93 were used for antigenic comparison. F171, a pooled mycelial-phase culture filtrate of 22 *C. immitis* strains, contains IDCF and IDTP activities and is routinely used in our laboratory to detect coccidioidal antibodies (19, 25). Heated F171 (60°C for 30 min), not reactive with the IgG of the IDCF reaction, is routinely used to detect differentially IgM precipitins by IDTP (19). CF pool 93, a pooled filtrate, is also reactive for both IDTP and IDCF and is used unheated in our laboratory for detection of coccidioidal antibodies by both IDCF and CF. Native coccidioidal chitinase/CF antigen was isolated from 48-h spherule-endospore culture filtrate by chitin affinity adsorption as described previously (10). *Histoplasma* and *Blastomyces* ID antigens (Meridian Diagnostics) were used to evaluate cross-reactivity.

ID. Antigenic reactivity was assessed by Ouchterlony double ID with pooled human sera. These sera are utilized in diagnostic testing by IDTP and IDCF for coccidioidal antibodies corresponding to IgM and IgG, respectively (18, 20). Reactivity was also evaluated with canine and equine sera that contained coccidioidal antibodies. The CF (IgG) titer of these nonhuman specimens was estimated by quantitative IDCF (19). The serum was added to designated wells of the ID plate neat or diluted in saline. The highest dilution yielding a visible precipitate was designated the titer. The serum was allowed to prediffuse for 2 h before the addition of antigen (8). Antigenic cross-reactivity was assessed with *Blastomyces* and *Histoplasma* ID antigens and antisera. These sera were not prediffused.

CF assay. Osmotic extracts, prepared from *E. coli* cells carrying either the pBluescript or pCTS 4-2A plasmid and their corresponding chitin affinity-purified fractions, were tested for complement-fixing activity by the modified Kolmer complement fixation test of Smith et al. (21, 22) in parallel with the reference coccidioidal antigen CF pool 93. The pCTS 4-2A extract, affinity-purified pCTS 4-2A (purified rCF antigen) fraction, and pBluescript extract were used at a concentration of 5 μg of protein per ml. Since presumably no protein would be recovered following chitin affinity adsorption-desorption of pBluescript extract, a volume equivalent to that of the affinity-purified rCF antigen solution was used.

EIA. Serum specimens were tested for reactivity by EIA using the affinity-purified rCF protein as the antigen. A total of 51 sera were tested for reactivity. Nineteen of these specimens were negative for IgM and IgG coccidioidal antibodies as determined by CF and by ID following eightfold concentration of the sera. Thirty-two of the specimens were positive for coccidioidal IgG antibodies as determined by ID and/or CF and ranged in titer from less than 1:2 (i.e., detectable only by ID) to more than 1:128. Six specimens demonstrated anticomplement activity or fixed complement incompletely. The titers of these specimens were determined by quantitative IDCF.

Immulon II 96-well round-bottomed microtiter plates (Dynatech, Chantilly, Va.) were coated with either 100 μl containing 0.12 μg of antigen in carbonate-bicarbonate buffer (0.05 M, pH 9.6) or 100 μl of buffer only and were incubated overnight at 4°C. For each serum tested, three antigen-coated wells and three buffer-only wells were prepared. The plates were washed with 0.05% Tween 20 in 0.1 M phosphate-buffered saline, pH 7.2 (PBST), three times, allowing each to soak 3 min. The wells were then blocked with 100 μl of PBST containing 10% normal goat serum (PBST-NGS) and were incubated at 37° for 1 h. The plates were then washed as previously described. One hundred microliters of the primary antibody (human serum), diluted 1:800 in PBST-NGS, was added to the wells, and the plates were incubated at 37°C for 1 h. Unreacted primary antibody was removed by washing as described above. Horseradish peroxidase-conjugated goat anti-human IgG (Cappell Laboratories, Durham, N.C.) was used as the secondary antibody diluted 1:10,000 in PBST-NGS. Aliquots of 100 μl were added to the wells, and the plates were incubated at 37°C for 1 h. Unreacted secondary antibody was removed by washing as described above. One hundred microliters of commercially prepared tetramethylbenzidine substrate (Sigma, St. Louis, Mo.) was added to each well. The plates were incubated in the dark for 30 min at room temperature. The enzymatic reaction was terminated by the addition of 100 μl of 2 N H_2SO_4. The A_{450} was read using a Bio-Rad model 3550 EIA Microplate reader, and air was used to blank the instrument. The net absorbance for each serum was calculated by subtracting the mean absorbance of the buffer-coated wells from the mean absorbance of the antigen-coated wells.

The cutoff value for designating a single specimen as EIA positive or negative was calculated from the mean net absorbance and the standard deviation of the negative serum sample. The value of 2 standard deviations (calculated using $n - 1$) was added to the value of the sample mean. Specimens with a net absorbance less than or equal to this value were designated negative, and specimens with a net absorbance greater than this value were designated positive.

Some of the specimens described above were also tested for reactivity by using a commercially available assay kit (Premier *Coccidioides* EIA; Meridian Diagnostics). Forty-three of the sera described above were used for comparison. Eleven were negative for coccidioidal antibodies by ID and CF, and 32 were positive. The Premier EIA was performed with the reagents and procedure provided by the manufacturer. Specimens were tested using both the anti-human IgM and anti-human IgG conjugates as recommended. The A_{450} was read with the reader described above. The negative control wells were used to blank the instrument. According to the manufacturer, a positive specimen yielded an A_{450} of 0.200 or greater. Readings between 0.150 and 0.199 were considered inconclusive, and specimens yielding readings of less than 0.150 were considered negative.

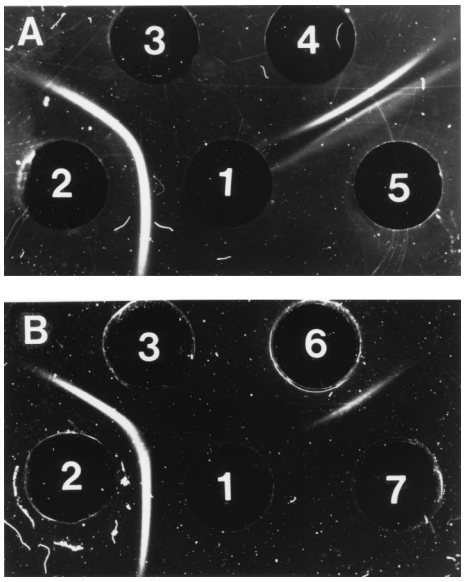

FIG. 2. ID reactivities of the affinity-purified rCF antigen with antisera to *Coccidioides* and *Histoplasma* (A) and *Coccidioides* and *Blastomyces* (B) spp. Well 1, affinity-purified rCF antigen; well 2, control *Coccidioides* IDCF-positive serum; well 3, CF pool 93 coccidioidal antigen; well 4, *Histoplasma* ID control antigen; well 5, *Histoplasma* ID control serum; well 6, *Blastomyces* ID control antigen; well 7, *Blastomyces* ID control serum.

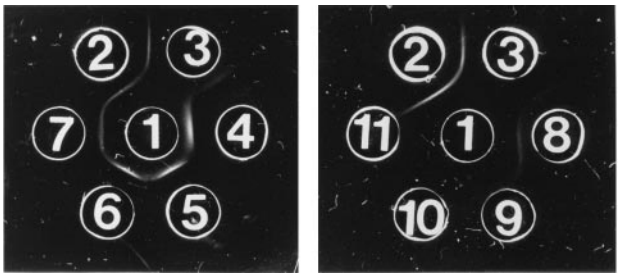

FIG. 3. Quantitative ID using a canine serum specimen. Well 1, affinity-purified rCF antigen; well 2, control *Coccidioides* IDCF-positive serum; well 3, CF pool 93 coccidioidal antigen. Wells 4 through 11 contained canine serum either neat or diluted as follows. Well 4, neat; well 5, 1:2; well 6, 1:4; well 7, 1:8; well 8, 1:16; well 9, 1:32; well 10, 1:64; well 11, 1:128.

RESULTS

Electrophoresis. The nonrecombinant (pBluescript) and recombinant (pCTS 4-2A) affinity-purified rCF antigen and the native affinity-purified coccidioidal chitinase/CF antigen were subjected to reducing SDS-PAGE (Fig. 1). The osmotic extracts prepared from *E. coli* containing either the nonrecombinant plasmid pBluescript (lane 1) or the recombinant plasmid pCTS 4-2A (lane 2) yielded numerous bands, including a major band at approximately 31 kDa. However, the affinity-purified rCF antigen, when electrophoresed (lane 3), yielded a major band at approximately 45 kDa and three faint bands at approximately 58, 40, and 31 kDa. The native affinity-purified coccidioidal chitinase/CF antigen (lane 4) migrated slightly slower than the rCF protein, at approximately 48 kDa. When electrophoresed under native nonreducing conditions, the affinity-purified rCF antigen migrated comparably with the native coccidioidal chitinase/CF antigen (data not shown).

ID. ID using pooled human sera and the affinity-purified rCF antigen produced an antigen-antibody precipitate which formed a line of identity with the precipitate band produced by the IDCF reference coccidioidal antigen CF pool 93 and human sera (Fig. 2). A minimum of 2.5 μg of protein per 40-μl well was required to produce a visible precipitation between the affinity-purified rCF antigen and the pooled human sera. This line of precipitation was not observed when the affinity-purified rCF antigen had previously been heated at 56°C for 30 min. There was no visible reaction between the affinity-purified rCF antigen and the IDTP control human serum (not shown). No cross-reactivity between the affinity-purified rCF antigen and either *Histoplasma capsulatum* or *Blastomyces dermatitidis* antisera was observed (Fig. 2). The affinity-purified rCF antigen yielded a precipitate with antibody-containing sera of canine and equine origin. The canine specimen had an antibody titer of 1:32 as determined by quantitative IDCF using the affinity-purified rCF antigen (Fig. 3), the titer detected by using the reference coccidioidal antigen CF pool 93. The equine serum antibody titer was similarly determined with the affinity-purified rCF antigen and this was 1:32, the titer previously determined with the reference coccidioidal antigen (not shown).

CF. Complement-fixing antibodies at a titer of 1:16 were detected in the human serum (Z4467) by using the affinity-purified rCF antigen at a concentration of 5 μg/ml (Table 1). This titer of 1:16 was detected for the same serum tested simultaneously with the reference coccidioidal antigen CF pool 93 at a protein concentration of 30 μg/ml. Only partial fixation was observed at a 1:2 dilution when the pCTS 4-2A whole (not purified) extract was used, and no fixation was observed when either of the pBluescript nonrecombinant fractions was used. No anticomplement activity was observed with any of the fractions. Additional serum specimens with various CF titers were tested for CF antibodies by using the affinity-purified rCF antigen and mycelial CF pool 93 antigen. The results obtained with three of these sera are presented in Table 2. In all cases, the titers determined with the two antigens differed by no more than 1 serial dilution.

EIA. Nineteen serum specimens negative for coccidioidal antibodies by ID and CF were assayed for reactivity by EIA by using the affinity-purified rCF antigen. The mean net absorbance (absorbance of antigen-coated well minus absorbance of buffer-coated wells) of this sample was 0.061, and the standard deviation (σ_{n-1}) was 0.050. The range was from 0.000 to 0.149. A cutoff value of 0.161 was calculated, yielding a 95% confidence interval about the mean.

The reactivities of 11 of the negative sera described above and 32 additional positive specimens were assayed and compared with the results obtained with the Meridian Premier EIA and the same specimens (Table 3). Using the above-described cutoff value, all 11 negative sera were correctly designated negative by the rCF EIA. Only one positive specimen (CF titer,

TABLE 1. Complement-fixing activity of recombinant products derived from coccidioidal cDNA in *E. coli*

Antigen	CF score[a]									
	Serum dilution								Serum control[b]	Antigen control[c]
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256		
CF pool 93[d]	4	4	4	3	0	0	0	0	0	0
Affinity-purified rCF antigen[e]	4	4	4	3	2	0	0	0	0	0
pCTS 4-2A osmotic extract[f]	2	0	0	0	0	0	0	0	0	0
Affinity-purified pBluescript osmotic extract[g]	0	0	0	0	0	0	0	0	0	0
pBluescript osmotic extract[h]	0	0	0	0	0	0	0	0	0	0

[a] 0, no fixation of complement; 2 and 3, partial fixation of complement; 4, complete fixation of complement.
[b] Serum plus complement.
[c] Antigen plus complement.
[d] Coccidioidal mycelial-phase culture filtrate; 8.5 μg of protein was used per tube.
[e] Recombinant CF antigen purified by affinity adsorption to chitin from osmotic extracts of *E. coli* containing recombinant plasmid pCTS 4-2A; 1.2 μg of protein was used per tube.
[f] Complete osmotic extract of *E. coli* containing recombinant plasmid pCTS 4-2A; 1.2 μg of protein was used per tube.
[g] Osmotic extract of *E. coli* containing the nonrecombinant pBluescript plasmid subjected to affinity adsorption to chitin; a volume equivalent to 1.2 μg of protein of the affinity-purified rCF antigen was used per tube.
[h] Osmotic extract of *E. coli* containing the nonrecombinant pBluescript plasmid; 1.2 μg of protein was used per tube.

TABLE 2. Comparison of complement-fixing activity obtained with the AP rCF and coccidioidal antigens

Serum specimen[a]	Antigen	CF score									
		Serum dilution									Serum control[b]
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	
Z4766	AP rCF[c]	4	3	0							0
	CF pool[d]	4	3	0							0
Z4853	AP rCF	4	4	4	4	2	0				0
	CF pool	4	4	4	4	3	0				0
Z4765	AP rCF	2	3	3	4	4	3	3	1	0	0
	CF pool	4	4	4	4	4	4	3	3	0	0

[a] Sera from three different patients.
[b] Anticomplement serum control; serum plus complement.
[c] Affinity-purified (AP) rCF antigen/chitinase.
[d] Coccidioidal mycelial CF pool 93 antigen.

1:2) was incorrectly designated negative (Table 4). The Premier EIA also correctly identified all 11 negative specimens; however, it incorrectly designated 5 positive specimens as negative (Table 4). Thus, while both the rCF EIA and the Premier EIA were 100% specific, the rCF EIA was more sensitive than the Premier EIA (96.9% and 84.4%, respectively).

Because the association between CF titer and severity of disease has been well established, it was necessary to examine the EIA reactivity of the serum with respect to CF titer. Figure 4 illustrates the reactivity as measured by absorbance in reference to CF titer. Data for serum specimens that either were anticomplementary or fixed complement incompletely are not included in the figure. The Meridian system appeared to group the sera into two clusters, one of sera with CF titers of less than 1:8 and another of sera with CF titers greater than 1:8. In contrast, the rCF EIA had a greater range of reactivity and the specimens appeared to cluster into three groups corresponding to low (titer, <1:4), medium (titer, >1:4 and <1:16), and high (titer, >1:16) CF titers.

DISCUSSION

The utility of detection and quantitation of CF antibodies for the diagnosis and evaluation of coccidioidal disease is well known (13, 21, 22). Previous work has shown that a 110-kDa protein which exhibits chitinase activity is the antigen that participates in both the IDCF and the CF reaction to detect coccidioidal antibody (10). The identification of the responsible protein has permitted the cloning of the corresponding gene and the production of an rCF antigen in *E. coli*. In previous work, we have presented and discussed the methods and rationale for cloning and expressing the coccidioidal CF antigen in *E. coli* (27). In this study, we evaluated the utility of the recombinant protein in conventional serological assays and compared its reactivity with those of currently used antigens.

TABLE 3. Comparison of serum reactivity as detected by two assay methods

Method	Absorbance					
	Negative sera (n = 11)			Positive sera (n = 32)		
	Mean	SD[a]	Range	Mean	SD[a]	Range
Affinity-purified rCF EIA	0.052	0.044	0.000–0.132	1.530	1.004	0.118–2.999
Meridian Premier EIA[b]	0.006	0.009	0.000–0.022	0.727	0.427	0.000–1.623

[a] Calculated by using $n - 1$.
[b] Absorbance is reported for IgG conjugate only.

TABLE 4. Discrepant specimens

Serum specimen	CF titer	Affinity-purified rCF EIA		Meridian EIA		
		Absorbance	Evaluation[b]	Absorbance		Evaluation[b]
				IgM	IgG	
Y0423	>1:4, <1:8	0.943	+	0.034	0.108	−
Y2605	1:2	0.510	+	0.004	0.082	−
W9654	1:2	0.118	−	0.000	0.000	−
X8800	>1:4, <1:8	1.176	+	0.000	0.137	−
X7128	<1:2	0.453	+	0.054	0.091	−

[a] Specimens were designated negative if the absorbance was ≤0.161 and positive if the absorbance was >0.161.
[b] Specimens were designated negative if the absorbance was <0.150, indeterminate if it was between 0.150 and 0.199, and positive if it was ≥0.200.

The affinity-purified rCF protein appears to share antigenic epitopes with the coccidioidal CF antigen, since a line of identity between the affinity-purified rCF antigen and the coccidioidal CF antigen is observed in the presence of IDCF-positive serum. The native coccidioidal CF antigen exists as a 110-kDa protein presumably composed of 48-kDa subunits (10, 19, 26). The affinity-purified rCF antigen was observed to migrate comparably to the coccidioidal affinity-purified CF antigen in nonreducing native PAGE and migrated as a prominent band at approximately 45 kDa under denaturing conditions. These observations suggest that the subunits produced in *E. coli* are capable of assembling and folding into a polymeric mature protein antigenically similar to the native coccidioidal CF antigen and migrating comparably in nondenaturing gels.

The affinity-purified rCF antigen in concert with CF-positive sera fixed complement and showed no anticomplement activity. The lack of CF by the extracts of *E. coli* cells containing the pBluescript (nonrecombinant vector only) control indicated that this reactivity was attributable to the recombinant antigen

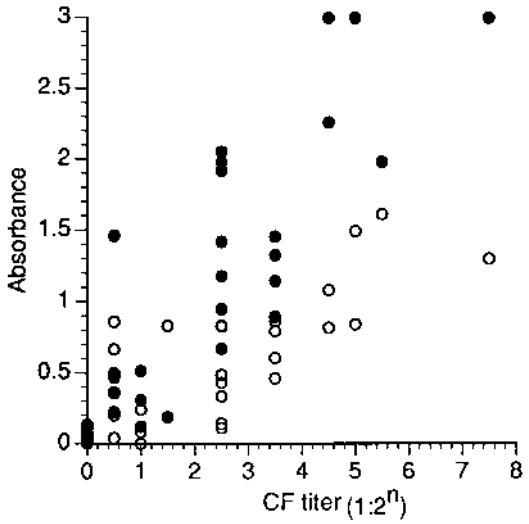

FIG. 4. Reactivity of the affinity-purified rCF EIA and the Meridian Premier *Coccidioides* EIA with respect to previously determined CF titer. Symbols: ○, Meridian Premier EIA; ●, affinity-purified rCF EIA.

and not due to a nonspecific *E. coli* antigen-antibody reaction. The reactivity of 5 μg of affinity-purified rCF antigen was comparable to that of 30 μg of protein of the coccidioidal CF pool 93 antigen though the coccidioidal antigen was not subjected to prior purification.

Some human sera and sera from other species, such as canid and equid species, may exhibit anticomplement activity, precluding the determination of an IgG titer by the CF assay. For these specimens, the titer can be estimated by quantitative IDCF, which has been shown to correlate well with the titer determined by CF at CF titers of 1:16 or greater (19). The affinity-purified rCF antigen was reactive with sera of canine and equine origin and by quantitative IDCF yielded an IgG titer comparable to that detected by quantitative IDCF using the coccidioidal CF pool antigen.

Cross-reactivity has been noted between some coccidioidal antigens and sera from patients with histoplasmosis or blastomycosis as well as other mycotic infections (3, 7, 9, 13, 14). Huppert et al. (7) identified eight antigenic components of coccidioidin that were shared with *Histoplasma* or *Blastomyces* spp. The affinity-purified rCF antigen showed no reactivity with anti-*Histoplasma* or anti-*Blastomyces* ID control sera, although limited samples of these sera were tested.

The EIA clearly offers a number of advantages over the IDCF and the CF assays. These include the small quantities of sera and antigen required, the degree of training required to perform and interpret the assay, and the potential for automation. However, the reactivity of sera as expressed by optical density readings in the EIA have not correlated well quantitatively with the CF titer, which provides prognostic information (14, 19, 21, 22). The affinity-purified rCF antigen has shown good sensitivity and specificity when used in the described EIA with single serum samples. Only one of 32 specimens was judged to be false negative. However, in the present study, 5 of 32 specimens that were positive for coccidioidal antibody by conventional tests were negative by the Meridian Premier *Coccidioides* EIA. When the reactivity was evaluated with respect to CF titer, the affinity-purified rCF antigen EIA showed a general trend of increasing EIA reactivity with respect to CF titer that appeared to cluster the sera into groups of low, medium, and high reactivity. With the Meridian EIA, the sera clustered into low- and high-reactivity groups. The optical density readings with the EIA using the affinity-purified rCF antigen in this small group of sera appeared more closely proportional to the CF titers than those obtained with the Premier test.

The ability to produce functional reactive recombinant antigens will surely have an impact on fungal serology. In addition to allowing development of new diagnostic methods and products, it will allow the production of diagnostic antigen so that results in different diagnostic laboratories can be standardized. The affinity-purified rCF antigen has been shown to be as reactive as the coccidioidal antigen in both the ID and CF assays. Additionally, the EIA using this antigen offers the possibility of providing both diagnostic and prognostic information. However, more sera including those from patients with heterologous fungal infections must be tested to evaluate adequately the recombinant antigen in serodiagnosis.

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