Blocking Effect of Rheumatoid Factor and Cold Agglutinins on Complement Fixation Tests for Histoplasmosis

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Received for publication 6 October 1975

The blocking effect of rheumatoid factor (RF) and cold agglutinins (CA) on the detection of complement-fixing (CF) antibodies for *Histoplasma capsulatum* using a mycelial (histoplasmin) and a yeast antigen was studied. Sera from 213 patients serologically positive for histoplasmosis were screened for the presence of RF or CA. CF antibodies to *H. capsulatum* in sera containing RF or CA were studied before and after removal of these factors (RF and CA) by treatment with dithiothreitol. Results suggest that RF or CA may interfere with the CF reaction to the yeast antigen of *H. capsulatum* but not to the mycelial antigen (histoplasmin).

Rheumatoid factor (RF) has been shown to interfere with complement fixation (CF) tests using viral and mycoplasma antigens (7, 9, 10; T. M. Brown, H. W. Clark, and J. S. Bailey, Abstr., Arthritis Rheum., p. 309, 1970). Attempts have been made to remove RF from sera by treatment with 2-mercaptoethanol, dithiothreitol (DTT), heat-aggregated human gamma globulin, and kaolin (5, 6, 10). A study by Stanford (10) has shown that after removal of RF a distinct rise occurs in CF antibody titers against herpes simplex, mumps, and morbilli viruses.

The CF test is the most widely used serological procedure for the diagnosis of pulmonary mycoses such as histoplasmosis and blastomycosis. In many instances of culture-proven fungal disease, however, the CF test may be negative (1, 8). In a study of 16 patients with culture-positive histoplasmosis, sera from 9 did not react with histoplasmin, sera from 6 did not react with the yeast antigen, and sera from 4 did not react with either antigen in the CF test (8). The many instances of serologically negative and low-titer-positive sera from patients with culture-proven histoplasmosis could be due to the presence of a blocking factor.

The report by Stanford (10) prompted us to study the effect of RF on CF antibody titers to histoplasmin and a yeast antigen by comparing control sera with sera serologically positive for *Histoplasma capsulatum* antigens and RF. Because cold agglutinins (CA) also appear to be immunoglobulin M-like macroglobulins, it was decided to study their effect on fungal CF tests as well.

MATERIALS AND METHODS

Sera. Sera from 213 patients serologically positive for histoplasmosis were screened for the presence of RF and CA. The sera were kept frozen at -60°C until used. Control sera consisted of 10 RF-positive sera obtained from the Department of Clinical Immunology, Mayo Clinic; 10 CA-positive sera from the blood bank, Mayo Clinic; 10 sera serologically nonreactive for histoplasmosis (mycelial and yeast antigens), RF, and CA; and 10 sera from patients with culture-proven histoplasmosis.

RF tests. RF was detected by the Hyland rheumatoid agglutination latex slide agglutination test before heat inactivation of sera. Titers of RF-positive sera were quantitated using the tube dilution technique as described in the Hyland RA kit brochure.

Tests for CA. The presence of CA was determined using the procedure of the American Association of Blood Banks (1). Control sera were tested using a twofold serial dilution, whereas 10-fold dilutions were used for test sera. The lowest titer for controls was 1:8, whereas the lowest positive reaction for the test sera was 1:10.

CF tests. Sera were heated to 56°C for 30 min before use in the CF tests. Sera were tested against histoplasmin (HKC-43, Center for Disease Control) and a *H. capsulatum* yeast antigen (A-811, Center for Disease Control) using the laboratory branch complement fixation micromethod advocated by the Center for Disease Control (4). Both DTT-treated and untreated sera from the same patients were tested simultaneously.

DTT treatment. Sera positive for RF on CA were treated with 0.02 M DTT in 0.2 M tris(hydroxymethyl)aminomethane buffer (pH 8.6). DTT (0.02 M) was made by dilution of Sputolysin (Calbiochem, Los Angeles) in tris(hydroxymethyl)aminomethane buffer. Equal volumes (0.1 ml) of serum and 0.02 M DTT were incubated at room temperature for 1 h.

After incubation, RF and CA tests were performed to ascertain that these factors had been removed.

RESULTS

The presence of RF and CA was determined in 213 test sera from patients serologically positive for histoplasmosis. The sera were reactive in the CF or immunodiffusion tests. Sixteen sera (7%) contained RF at titers of $\geq 1:20$, whereas six sera (2.6%) demonstrated CA in titers of $\geq 1:10$.

CF tests were performed on these sera before and after treatment with DTT. Only 1 of 16 RF-positive sera had a demonstrable CF titer to histoplasmin (Table 1). This titer did not change after treatment with DTT. The CF antibody titer to the *H. capsulatum* yeast antigen was constant in 6 of 16 instances. Ten of the sera demonstrated at least a twofold increase in titer, and seven of those increased fourfold or more. The titer of one sample rose from negative ($<1:2$) before treatment with DTT to 1:32 after treatment (16-fold increase). Four other sera showed an increase in titer from $<1:2$ to 1:8 (fourfold increase).

The six sera positive for CA were processed in the same manner. Table 2 shows that no change in titer occurred when histoplasmin was used. However, after DTT treatment the *H. capsulatum* yeast CF antibody titer increased in four of six instances. Three sera that were nonreactive before removal of the CA demonstrated fourfold, eightfold, and 16-fold rises in titer. The remaining serum sample was

TABLE 1. *Histoplasma* CF titers in sera containing RF before and after treatment with DTT^a

Antigen	Before DTT treatment ^b		Change in titer after DTT treatment (-fold) ^c			
	Antigen titer	No. of samples	0	+2	+4	+16
Yeast	$<1:2$	9	4		4	1
	1:8	1			1	
	1:16	3	1	1	1	
	1:32	1	1			
	1:64	2		2		
Histoplasmin	$<1:2$	15	15			
	1:16	1	1			

^a Sera contained antibodies to at least one *Histoplasma* antigen.

^b RF test was positive in each sample (titer, $\geq 1:20$).

^c RF test was negative in each sample. No titers showed an eightfold increase after DTT treatment.

TABLE 2. *Histoplasma* CF titers in sera containing CA before and after treatment with DTT^a

Antigen	Before DTT treatment ^b		Change in titer after DTT treatment (-fold) ^c			
	Antigen titer	No. of samples	0	+4	+8	+16
Yeast	$<1:2$	4	1	1	1	1
	1:16	1		1		
	1:64	1	1			
Histoplasmin	$<1:2$	6	6			

^a Sera contained antibodies to at least one *Histoplasma* antigen.

^b Test for CA positive in all samples (titer, $\geq 1:10$).

^c Test for CA negative in all samples. No two- or 16-fold increases in titer after DTT treatment were noted.

TABLE 3. *Histoplasma* CF titers in sera from patients with cultural evidence of histoplasmosis before and after treatment with DTT^a

Antigen	Before DTT treatment		Change in titer after DTT treatment (-fold)		
	Antigen titer	No. of samples	-2	0	+2
Yeast	$<1:2$	1		1	
	1:8	2		2	
	1:16	3		2	1
	1:64	4		4	
Histoplasmin	$<1:2$	5		5	
	1:8	1		1	
	1:64	4	1	3	

^a Sera did not contain CA or RF.

positive before DTT treatment, and the titer increased from 1:16 before treatment to 1:64 after treatment (fourfold increase).

Control sera from 10 patients with culture-proven histoplasmosis which were nonreactive for RF and CA showed only minor changes (Table 3). One serum sample showed a rise in titer from 1:16 to 1:32, and the titer of another decreased from 1:64 to 1:32.

Ten RF-positive control sera were tested for the presence of CF antibodies before and after removal of the RF, and in no instance did the titers to either of the *H. capsulatum* antigens rise. After DTT treatment, the RF was not detectable.

Ten sera positive for CA gave the same results, with no rise in titer after DTT treatment; CA were not detectable.

DISCUSSION

Several investigators have shown that sera containing RF can yield falsely depressed CF titers (10; N. J. Zvaifler and K. J. Bloch, *Abstr., Arthritis Rheum.*, p. 127, 1962; F. R. Schmid and M. D. Rocha, *Abstr., Arthritis Rheum.*, p. 508, 1968; J. D. Smiley and H. L. Casey, *Abstr., Arthritis Rheum.*, p. 698, 1969; T. M. Brown, H. W. Clark, and J. S. Bailey, *Abstr., Arthritis Rheum.*, p. 309, 1970). It would appear that this interference occurs by competition between the RF and complement for the antigen-antibody complex (6, 7, 10). The inhibition by RF-positive sera can be removed by several methods. Gordon and Lapa (5) have shown that DTT can degrade and remove RF from sera by splitting the macroglobulin into 7S monomers just as does 2-mercaptoethanol (5, 7). The present study supports their work, in that RF was removed from all positive sera, including sera with high titers, using DTT.

Many investigations of RF have been concerned with its effect on viral CF tests. One such study has been done with mycoplasma (T. M. Brown, H. W. Clark, and J. S. Bailey, *Abstr., Arthritis Rheum.*, p. 309, 1970). This report is the first that examines the blocking effect of RF on fungal CF tests. Supporting the earlier work with viral antigens are our findings that RF-positive sera may exert a blocking effect on *H. capsulatum* yeast CF antibody titers and thereby yield slightly depressed titers. Other reports have indicated an inverse correlation between RF titers and CF antibody titers (10; J. D. Smiley and H. L. Casey, *Abstr., Arthritis Rheum.*, p. 698, 1969). This relationship did not appear to occur in our study, although we did not specifically investigate the problem.

Our investigations included a determination of possible interference with fungal CF tests by CA. This was included because CA appear to be primarily immunoglobulin M-like macroglobulins (3) and could possibly act in a manner analogous to RF. Indeed, CA were shown to inhibit the *H. capsulatum* yeast CF antibody titers, and this effect could be alleviated by treatment with DTT.

In addition to studying the effects of RF and CA on CF tests for histoplasmosis, we examined sera from 23 patients serologically positive for blastomycosis for the presence of RF and CA. None contained RF or CA. In addition, sera from seven patients with culture-proven blastomycosis were tested and, likewise, were negative for RF and CA. It was hoped that these factors might be present and could help explain the many instances of serologically

negative blastomycosis patients. However, the lack of RF and CA in those sera available precluded any possible study.

Addition of DTT to sera from patients with culture-proven histoplasmosis, but lacking RF or CA, did not alter CF antibody titers. This indicates that the CF titers obtained after DTT treatment for removal of RF or CA are the true antibody titers to *H. capsulatum* and are not falsely elevated.

Stanford (10) and others (6, 9) have proposed that RF-positive sera or other sera suspected of yielding a false-negative CF test should be pretreated to remove the blocking effects of such sera. Of 213 sera screened for RF and CA in this study, 16 (7%) demonstrated RF and 6 (2.6%) contained CA. These values are consistent with incidences among the normal population. Eight of these 22 sera demonstrated a change in titer from nonreactive to 1:8, considered by some to be presumptive evidence of histoplasmosis (8). However, results obtained in this laboratory indicate that a titer of 1:16 is necessary to make a presumptive diagnosis of histoplasmosis. Using this criterion, 5 of 22 RF- or CA-positive sera demonstrated a clinically significant rise in titer (2 RF-positive sera and 3 CA-positive sera). These 5 were among 213 specimens serologically positive for histoplasmosis and represented less than 0.2% of all sera tested for fungal serology. The results of this study do suggest that the presence of RF or CA may interfere with *H. capsulatum* yeast CF antibody titers.

ACKNOWLEDGMENTS

We would like to thank Howard F. Taswell and Harold D. Markowitz for providing CA-positive and RF-positive control sera, respectively. Thanks are also extended to Bevelyn E. Olson and Kathryn Kurek for technical assistance.

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