JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1984, p. 1171–1173
0095-1137/84/121171-03$02.00/0

Vol. 20, No. 6

Evaluation of a C-Reactive Protein Latex Agglutination Detection Test with Sera from Patients with Sexually Transmitted Diseases

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Received 5 June 1984/Accepted 7 September 1984

A total of 149 sera, including 79 pre- and posttreatment sera from 33 patients with disseminated gonococcal infections, 18 from patients with uncomplicated gonococcal infections, 6 from patients with pelvic inflammatory disease, 4 from patients with genital *Chlamydia trachomatis* infections, and 42 from normal volunteers, were examined for C-reactive protein with a latex agglutination C-reactive protein detection kit (Difco Laboratories, Detroit, Mich.). Results were quantitated with LC-Partigen C-reactive protein radial immunodiffusion plates (Calbiochem-Behring, La Jolla, Calif.). Positive latex agglutination results were observed in all of the pretreatment sera and some of the posttreatment sera of patients with disseminated gonococcal infections and in two sera from patients with pelvic inflammatory disease, which corresponded to quantitative C-reactive protein levels in the radial immunodiffusion plates. C-reactive protein levels were not detectable in the serum samples from normal volunteers or patients with uncomplicated gonococcal infections or genital chlamydial infections. Positive latex agglutination occurred as early as 20 s in sera with high C-reactive protein levels, and all positive results were observed within 90 s of the 3-min test limit. Positive latex test results were obtained with C-reactive protein levels as low as 1 mg/dl (10 μg/ml).

During the course of inflammatory stimulation, the acute-phase response in a host exhibits a characteristic and noticeable rise in the concentration of numerous plasma proteins. These changes in the plasma protein profile may have an effect on other nonspecific, indirect responses, such as the erythrocyte sedimentation rate (ESR) and the plasma viscosity (17). ESR measurement is a commonly used method for monitoring inflammatory disease (17), and in the case of pelvic inflammatory disease or disseminated gonococcal infection, measurement of ESR is an adjunct in clinical diagnosis (2–4, 8, 14). However, changes in ESR and plasma protein are not rapid and usually occur over a period of days (17). Unlike those of other plasma proteins, C-reactive protein (CRP) concentration not only rises above its normal level of 580 ng/ml faster than any other acute-phase proteins, but it will also drop rapidly towards normal (17). Since it is a trace constituent of normal plasma and can increase several hundredfold within a period of hours, CRP is probably a superior indication of an inflammatory response (1, 6, 9, 17, 21). In recent years, the biological activity of CRP is being defined (5, 15, 18) and the role that it may play in the mechanism of resistance to infection is progressively being elucidated (10–13).

Because CRP is an indicator of inflammation and remains elevated during that response, a test system that could rapidly detect the presence of CRP would be advantageous. The latex-agglutination (LA) kit for CRP detection (Difco Laboratories, Detroit, Mich.) is a rapid, sensitive, and simple test that requires a maximum of 3 min. Each kit contains negative and positive CRP control sera, and the reagents may be mixed by gentle hand rocking or by being placed on a bench-top rotator.

In this study we evaluated the CRP LA test and compared it with a quantitative radial immunodiffusion (RID) assay to demonstrate its speed and sensitivity.

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MATERIALS AND METHODS

The test serum was obtained from five different groups. The first group consisted of 42 normal serum samples drawn from 14 female volunteers before, during, and after their menstrual cycles. The second group contained 79 serum samples taken at the initial visit and, when there were return visits, serum samples at 1 to 3 weeks posttreatment from patients with confirmed cases of disseminated gonococcal infections (DGI). The third group contained 18 serum samples from patients with uncomplicated gonococcal infections (UGI). The fourth group contained six serum samples from patients with confirmed cases of pelvic inflammatory disease (PID), and the fifth group contained four serum samples from patients with confirmed cases of genital *Chlamydia trachomatis* (Ct) infection. Patients were confirmed as positive for DGI by cultures of blood and joint fluid that were positive for *Neisseria gonorrhoeae*. The PID serum specimens were obtained from patients admitted to a large urban hospital in Atlanta, Ga. during a study of PID. All diagnoses were confirmed by culdocentesis, laparoscopy, or both. All patients had a temperature of 38°C or greater on admission (20). Patients were confirmed as positive for UGI by cervical cultures that were positive for *N. gonorrhoeae*. Patients were confirmed as positive for Ct infection with infected McCoy tissue culture cells by iodine staining of inclusion bodies or immunofluorescence. Unless indicated otherwise, all serum samples were obtained before administration of antibiotics.

The LA test was performed by diluting the serum specimens 1:5 and 1:50 in the glycine-buffered saline supplied with the test kit. The positive and negative control sera, also supplied, were used undiluted. All procedures were performed as described in the instructions of the manufacturers.

With a Pasteur pipette, one drop (ca. 50 μl) of each diluted serum specimen was placed on each section of the reaction slides, and with the dropper supplied with the reagent bottle, one drop (ca. 50 μl) of the anti-CRP latex reagent was added

TABLE 1. Detection of C-reactive protein in serum samples from patients with sexually transmitted diseases as measured by LA and RID assay

Serum	Presence of CRP (no. of positive samples/total)		
	RID	LA (1:5 dilution)	LA (1:50 dilution)
DGI			
Initial visit	33/33	33/33	14/33
1st Week posttreatment	11/20	11/20	3/20
2nd Week posttreatment	3/20	3/20	1/20
3rd Week posttreatment	2/6	2/6	0/6
PID	2/6	2/6	ND[a]
UGI	0/18	0/18	ND
Ct	0/4	0/4	ND
Normal	0/42	0/42	0/42

[a] ND, Not done.

to each. The glass slide was then rocked or rotated for up to 3 min. Very strong reactions, exhibiting large agglutinated clumps, were graded 4+, which was the type of reaction observed with the supplied positive control serum. Strong reactions were graded 3+, moderate reactions were graded 2+, and weak reactions were graded 1+. A negative reaction showed no agglutination and was compared with the lack of reactivity of the latex reagent with the negative control serum.

For quantitation of CRP levels in the test sera, LC-Partigen CRP RID plates were obtained from Calbiochem-Behring (La Jolla, Cal.). RID assay was performed by placing 20 μl of each test serum and 20 μl of the supplied reference serum dilutions (1:1, 1:2, 1:4) into the wells of the agarose anti-human CRP plates. At 72 h, the diameter of each reaction was measured with dissecting calipers and recorded in millimeters. Concentrations of CRP were determined from a standard curve constructed from data obtained with known concentrations of CRP in the reference sera.

RESULTS

The results of the LA tests and RID assay correlated well, especially at the 1:5 dilution (Table 1). Negative LA and RID results were obtained with the normal volunteer sera. The results were also negative by both test systems with the sera obtained from both the Ct infections and the UGI. Only two of the six serum specimens obtained from patients with PID, showed a 1+ reaction with the LA, and the RID assay for these samples showed an extremely small precipitin zone that was barely measurable. All of the DGI sera obtained at the initial clinic visit showed positive results in both the LA test (1:5 dilution) and the RID assay. RID values ranged from the highest value of 21.0 mg/dl to the lowest value of 2.1 mg/dl. Likewise, the LA patterns were 3+ to 4+ reactions within the low to high values. The DGI sera showing the highest initial values demonstrated progressively lower RID values with the serum samples from the second week posttreatment and in the few samples obtained in the third week posttreatment, the CRP levels were undetectable by either test system. Likewise, the sera with lower initial CRP levels were negative in both test systems by the second week posttreatment.

DISCUSSION

This study has demonstrated a 100% agreement of the Difco LA test with a commercial RID assay for CRP. The LA test did not show nonspecific agglutination or false-positive reactions, within the limits of testing done in our laboratory. This statement holds true even though we used the LA test, in some cases, after the reagent expiration date.

We used serum samples taken from normal female volunteers before, during, and after menses because we were not sure whether the commencement of menses would cause CRP to be elevated in our test results. Although an increase in ESR is not necessarily simultaneous with an increase in CRP (7, 17), physiological changes in the human body, e.g., pregnancy menstruation, or anemia, can show an increased ESR without an elevated CRP (19). Serum samples used in the RID assay were used undiluted. In the LA test, serum samples were tested at 1:5 and 1:50 serum dilutions. In most cases, positive LA results occurred within 20 to 45 s, but some weaker reactions required 60 to 90 s. However, all LA test results were easily obtained before the 3-min limit stipulated by the manufacturer. Compared with the known concentration of the CRP control sera supplied in the RID assay and the quantitation of each serum sample, the LA test for CRP detection could demonstrate a 1+ reaction with a CRP concentration of less than 1 mg/dl (10 μg/ml). The LA test was shown to be a fast, simple, specific test for detecting CRP compared with the RID assay, which required 60 to 72 h.

The elevation of CRP is a nonspecific response, much the same as an elevated temperature or an increased ESR. Each of these phenomena is affected by an inflammatory response which can be induced by numerous factors (2–4, 8, 14). Because CRP is a nonspecific response, we are not suggesting that detection of CRP should be used solely to diagnose DGI. But since CRP may rise more rapidly or fall more rapidly than other nonspecific responses (1, 6, 9, 17, 21), detecting elevated CRP may be a helpful adjunct for early confirmation. Additionally, Penner has suggested that CRP may be useful for therapy as a monitor during the course of certain diseases (16). A rapid detection system, such as the latex agglutination test, may be helpful then in following the treatment response of patients with DGI. We are unable to explain fully why there was not a significant elevated CRP level in two PID patients and absent in the rest. Genital Ct infections and UGI are localized and may not induce an inflammatory response that would be acknowledged by a systemic increase in CRP. The systemic infection in DGI and the accompanying joint involvement demonstrated an elevated CRP in all of the DGI sera obtained. Perhaps, since PID is not a systemic involvement, it may be exhibited like the localized Ct infections or UGI with regard to elevated CRP. It is quite obvious that more PID serum specimens need to be examined to determine what relationship exists between CRP and PID. Additionally, since the binding affinity of CRP to gonococci is quite low (12), it is unlikely, in this case that CRP, with its ability to bind complement, would aid as a defense mechanism in the host. Consequently, the detection of CRP in DGI can probably only serve as an indication of the inflammatory response.

ACKNOWLEDGMENTS

We thank A. L. Lane and M. M. Moon of Difco Laboratories for supplying the latex agglutination test kits.

LITERATURE CITED

1. **Gotschlich, E.** 1982. Binding of C-reactive protein to C-carbohydrate and PC-substituted protein. Ann. N.Y. Acad. Sci. **389**:163–168.
2. **Handsfield, H. H.** 1977. Clinical aspects of gonococcal infections., p. 58–79. *In* R. B. Roberts (ed.), The gonococcus. John Wiley & Sons, Inc., New York.

3. **Holmes, K. K., G. W. Counts, and H. N. Beatty.** 1971. Disseminated gonococcal infection. Ann. Intern. Med. **74**:979–993.
4. **Jacobson, L., and Lars Westrom.** 1969. Objectivized diagnosis of acute pelvic inflammatory disease. Am. J. Obstet. Gynecol. **105**:1088.
5. **Kaplan, M. H., and J. E. Volanakis.** 1974. Interaction of C-reactive protein complexes with the complement system. J. Immunol. **112**:2135–2147.
6. **Kushmer, J.** 1982. The phenomenon of the acute phase response. Ann. N.Y. Acad. Sci. **389**:39–48.
7. **Mallya, R. K., F. C. de Beer, H. Berry, E. D. B. Hamilton, B. E. W. Mace, and M. B. Pepys.** 1982. Correlation of clinical parameters of disease activity in rheumatoid arthritis with serum concentration of C-reactive protein and erythrocyte sedimentation rate. J. Rheumatol. **9**:224–228.
8. **Mardh, P.-A., T. Buchanan, P. Christensen, D. Danielsson, I. Lind, and K. Reimann.** 1978. Comparison of four serological tests for detection of gonococcal antibodies in patients with complicated gonococcal infections, p. 377–381. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
9. **Mold, C., T. W. Du Clos, S. Nakayama, K. M. Edwards, and H. Gewurz.** 1982. C-reactive protein reactivity with complement and effects on phagocytosis. Ann. N.Y. Acad. Sci. **339**:251–259.
10. **Mold, C., K. M. Edwards, and H. Gewurz.** 1982. Effect of C-reactive protein on the complement-mediated stimulation of human neutrophils by *Streptococcus pneumoniae* serotypes 3 and 6. Infect. Immun. **37**:987–992.
11. **Mold, C., S. Nakayama, T. S. Holzer, H. Gewurz, and T. W. Du Clos.** 1981. C-reactive protein is protective against *Streptococcus pneumoniae* infection in mice. J. Exp. Med. **154**:1703–1708.
12. **Mold, C., C. P. Rodgers, R. L. Kaplan, and H. Gewurz.** 1982. Binding of human C-reactive protein to bacteria. Infect. Immun. **38**:392–395.
13. **Nakayama, S., C. Mold, H. Gewurz, and T. W. Du Clos.** 1982. Opsonic properties of C-reactive protein *in vivo*. J. Immunol. **128**:2435–2438.
14. **O'Brien, J. P., D. L. Goldenberg, and P. A. Rice.** 1983. Disseminated gonococcal infection: a prospective analysis of 49 patients and a review of pathophysiology and immune mechanisms. Medicine **62**:395–406.
15. **Olivera, E. B., E. C. Gotschlich, and T. Y. Liu.** 1980. Comparative studies on the binding properties of human and rabbit C-reactive proteins. J. Immunol. **123**:1396–1402.
16. **Penner, E.** 1981. C-Reactive protein, p. 448–449. *In* F. Milgrom, C. J. Abeyounis, and K. Kano (ed.), Principles of immunological diagnosis in medicine. Lea & Febiger, Philadelphia.
17. **Pepys, M. B.** 1979. Acute phase phenomena, p. 85–89. *In* A. S. Cohen (ed.), Rheumatology and immunology. Grune and Stratton, New York.
18. **Pepys, M. B.** 1981. C-reactive protein fifty years on. Lancet **i**:653-656.
19. **Seiverd, C. E.** 1983. Hematology for medical technologists, 5th ed. Lea & Febiger, Philadelphia.
20. **Thompson, S. E., W. D. Hager, K. H. Wong, B. Lopez, C. Ramsey, S. D. Allen, M. D. Stargel, C. Thornsberry, B. B. Benigno, J. D. Thompson, and J. A. Shulman.** 1980. The microbiology and therapy of acute pelvic inflammatory disease in hospitalized patients. Am. J. Obstet. Gynecol. **136**:179–186.
21. **Volanakis, J. E.** 1982. Complement activation by C-reactive protein complexes. Ann. N.Y. Acad. Sci. **389**:235–249.