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Evaluation of Passive Bacterial Agglutination for the Diagnosis of Typhoid Fever

T. JACOB JOHN,* K. SIVADASAN, AND BETTY KURIEN

Department of Virology and Immunology, Christian Medical College Hospital, Vellore, India

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We evaluated the reliability of a passive bacterial agglutination test to detect *Salmonella typhi* somatic antigen(s) in the sera of patients with typhoid fever. It was positive in 32 of 33 bacteriologically proven typhoid fever cases. Among 13 patients with a presumptive diagnosis of typhoid fever, 11 were positive by passive bacterial agglutination. The serum of one patient with paratyphoid A was also positive. Among 50 febrile patients without typhoid fever, one was persistently positive during the course of illness; 49 were negative. The sensitivity, specificity, and accuracy indices of the passive bacterial agglutination test were over 95%. The positive and negative predictive values were 94 and 98%, respectively.

Recently we have described a simple and rapid test for the diagnosis of typhoid fever based on the agglutination of antibody-coated staphylococci by *Salmonella typhi* somatic antigen(s) in the serum of patients (9). The term "coagglutination" has been used for this procedure when the antigen is particulate, and the term "passive bacterial agglutination" (PBA) has been used when the antigen is soluble (6, 7). The sensitivity, specificity, and accuracy of PBA for the diagnosis of typhoid fever were evaluated in 97 febrile patients, and the results presented below confirm that it is a reliable test.

MATERIALS AND METHODS

Antiserum was raised in rabbits by the intravenous injection of a nonmotile, capsule (Vi)-negative strain (Colindale no. 8393) of *S. typhi* treated with alcohol and acetone (2, 9). The antiserum was absorbed with Formalin-treated *Salmonella paratyphi* A, *Escherichia coli*, and *Pseudomonas aeruginosa* because it was found to agglutinate these organisms (5). After absorption, these organisms were not agglutinated by the antiserum.

Formalin-stabilized cells of *Staphylococcus aureus* bearing protein A (Cowan I strain) were sensitized by mixing a 10% (vol/vol) suspension in phosphate-buffered saline (PBS [0.03 M PBS containing 0.15 M NaCl; pH 7.2]) with the absorbed *S. typhi* antiserum in a proportion of 10:1 (vol/vol) and incubating the mixture at ambient temperature for 2 h (6, 7). The cells were then washed and suspended to 1% (vol/vol) in PBS; this mixture is designated PBA reagent. As the control reagent, staphylococcal cells were similarly treated with preimmune rabbit serum.

Formalinized *S. typhi* cells were ground with powdered glass in a sterile mortar cooled in an ice bath, and an aqueous extract was made and clarified by centrifugation; the supernatant is designated soluble *S. typhi* antigen (10).

The control and PBA reagents were tested by mixing 50 μl of each with an equal volume of the soluble antigen and rotating the mixture manually for 2 min. The PBA reagent was agglutinated, but the control reagent was not.

Acute-phase blood samples were collected from 97 febrile patients at 3 to 18 (mean 7.8) days after onset of fever. Second blood samples were collected from 44 patients at 3 to 17 (mean 8.1) days later. The sera were coded and sent to the laboratory where they were stored at 4°C and tested in batches of 10 sera. The patients belonged to three diagnostic groups. The first group (33 patients) had confirmed typhoid fever with *S. typhi* grown in blood culture. The second group (13 patients) had a presumptive diagnosis of typhoid fever based on compatible clinical symptoms, but blood culture did not grow *S. typhi*. Both these groups were treated with antibiotics appropriate for typhoid fever. The third group (51 patients) had other diseases of confirmed etiology (13 patients) or short febrile illnesses of undiagnosed origin (38 patients). The specific diagnoses included culture-proven paratyphoid A, staphylococcal septicemia, pneumococcal meningitis, and shigella dysentery in four patients; aseptic meningitis, malaria, microfilaremia, or cirrhosis of the liver in four patients; tuberculosis in two patients; and urinary tract infections in three patients.

The mean intervals between the onset of fever and the collection of the acute-phase serum sample were 9.3, 9.2, and 5.9 days in the first, second, and third groups of patients, respectively. The mean intervals between the collection of the first and second serum samples were 9.3, 8.6, and 4.8 days in the three groups.

All sera were screened for the presence of staphylococcal agglutinins in 2-min slide agglutination tests with 1% (vol/vol) Formalin-stabilized staphylococcal cells in PBS. Positive sera were absorbed with 10% (vol/vol) stabilized staphylococcal cells. All sera were then tested for agglutination with the PBA and control reagents, as described earlier.

The indices of sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of the PBA test were calculated according to the formulae given below (4). The standards against which PBA was evaluated included the 33 culture-proven typhoid fever cases and the 51 cases defined as not typhoid fever. Therefore, only the results of PBA test on the acute-phase serum samples of patients in groups 1 and 3 were included in this analysis. The indices were calculated as follows: sensitivity, $[a/(a + c)] \times 100$; specificity, $[d/(b + d)] \times 100$; accuracy, $[(a + d)/(a + b + c + d)] \times 100$; positive predictive value, $[a/(a + b)] \times 100$; negative predictive value, $[d/(c + d)] \times 100$; where a is the number of true-positive samples, b is the number of false-positive samples, c is the number of false-negative samples, and d is the number of true-negative samples.

RESULTS

Agglutination of the PBA reagent by serum from a patient with typhoid fever and the absence of agglutination of the

* Corresponding author.

control reagent in the presence of the same serum are illustrated in Fig. 1.

Among the 33 acute-phase serum samples from patients with confirmed typhoid fever, 32 (97%) were positive for *S. typhi* antigen in PBA. In the patient with a negative PBA, the interval between onset of fever and collection of blood was 17 days. Of the 13 acute-phase sera from patients with presumptive typhoid fever, 11 (85%) were positive. Among the 51 first sera from patients with other febrile conditions, 2 (4%) were positive. One of them had *S. paratyphi* A grown in blood culture and the second had a short fever of undiagnosed etiology.

The sensitivity, specificity, and accuracy indices of PBA were 97, 96, and 95%, and the positive and negative predictive values were 94 and 98%, respectively.

The results of PBA on 44 second serum samples were as follows. Among the 34 samples from the confirmed and presumptive typhoid fever groups, 11 (32%) were positive. Among the same patients, 32 (94%) first samples had been positive. In the 21 patients in whom antigen clearance was shown, the intervals between the onset of fever and the collection of the second serum samples were 11 to 28 (mean, 18.1) days. Of the 10 second samples of the non-typhoid patients, 2 were positive. In each patient of groups 1, 2, or 3 in whom the second sample was positive, the first also was positive.

We have not used the results of Widal test in this study, on account of the frequency of false-positive and false-negative results. Among the 33 culture-proven typhoid fever patients, 29 were tested but only 16 had titers of 1:80 or more against H or O antigen. Among the 13 patients with a presumptive diagnosis of typhoid fever, 12 were tested, and 8 had similar significant titers. These 24 Widal-positive patients were PBA positive in their acute-phase sera.

DISCUSSION

S. typhi has three main antigenic components, namely, somatic (O), flagellar (H), and capsular (Vi). The antiserum used in this study was O specific, since it was raised against a nonmotile and Vi-negative strain and it did not react with *S. typhi* H or Vi antigen. Therefore, we presume that our test detects somatic antigen(s). The somatic antigen complex has many determinants. Some of them are antigenically related to those of other members of the genus *Salmonella* or the family *Enterobacteriaceae*. As the antiserum was found to agglutinate three species of bacteria including *S. paratyphi* A, it was absorbed with them until there was no further agglutination. Yet the serum of one patient with paratyphoid A agglutinated the PBA reagent. When we prepared *S. paratyphi* A-soluble antigen, it also agglutinated the PBA reagent, indicating that the antigen(s) involved in this reaction may be deep-seated in the organism.

The sensitivity, specificity, and accuracy indices of PBA were over 95%, indicating that it is a reliable test for typhoid fever. The advantages of PBA include rapidity, simplicity, and specificity. Undoubtedly, PBA is more reliable than Widal test for the diagnosis of typhoid fever. However, it should be further evaluated in other bacteremic illnesses to determine the frequency of false-positive reactions before its routine use can be recommended.

Antigen clearance was demonstrated during the course of illness in the majority of typhoid fever patients tested. However, we have not investigated the dynamics of antigen clearance in terms of time or antibiotic therapy. It is to be noted that the mean interval between onset of fever and the time of demonstration of antigen clearance was 18 days and that, in the culture-proven typhoid fever patient in whom the acute-phase serum was negative by PBA, the sample was collected 17 days after onset of fever.

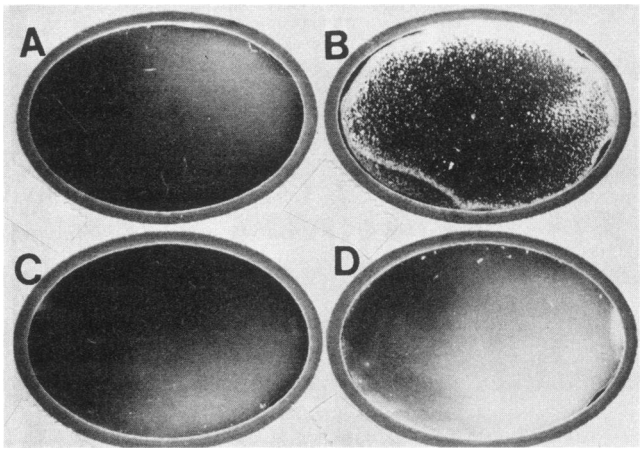

FIG. 1. PBA test. (A) Serum of typhoid fever patient and control reagent; no agglutination. (B) Same serum and PBA reagent showing agglutination. (C) Negative serum and control reagent; no agglutination. (D) Negative serum and PBA reagent; no agglutination.

The two false-positive results are noteworthy. One patient had paratyphoid A. Although the PBA result was false-positive by definition, it is likely that the agglutination was due to shared antigenic determinants between *S. typhi* and *S. paratyphi* A. For the second false-positive reaction, the reason remains unexplained. This patient did not have a bacteremic illness and was not treated with antibiotics. Here the test was indeed false-positive and this should caution us from placing complete confidence in this procedure. More work needs to be done in identifying the relevant antigen(s) detected in the sera of patients with typhoid fever before such false-positive reactions could be eliminated.

Others have investigated the frequency of antigenemia and antigenuria of different specificities in typhoid fever. The detection of D, d, and Vi antigens in urine using PBA has been reported; although this test was highly sensitive, false-positive reactions were frequent (8). More recently enzyme-linked immunoassay and PBA have been described for the detection of Vi antigen in the urine of typhoid patients (1). Both tests are of low sensitivity and specificity (11). Counterimmunoelectrophoresis has been reported to be a suitable screening method for the detection of *S. typhi* antigen and antibody (3, 10). In our preliminary studies, the sensitivity of counterimmunoelectrophoresis was found to be low (9). All sera tested by PBA in this investigation have also been tested in counterimmunoelectrophoresis for a comparative evaluation, which will be presented in another communication.

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