Comparison of Staphylococcal Coagglutination, Latex Agglutination, and Counterimmunoelectrophoresis for Bacterial Antigen Detection

M. C. THIRUMOORTHI AND ADNAN S. DAJANI*
Division of Infectious Diseases, Children’s Hospital of Michigan and Department of Pediatrics, Wayne State University School of Medicine, Detroit, Michigan 48201

Received for publication 6 September 1978

Soluble antigens of Haemophilus influenzae type b, Streptococcus pneumoniae, Neisseria meningitidis, and group B streptococcus were looked for in cerebrospinal fluid, serum, and urine by using the staphylococcal coagglutination test, latex agglutination test, and counterimmunoelectrophoresis. The staphylococcal coagglutination and latex agglutination tests were more sensitive than counterimmunoelectrophoresis in identifying antigens of H. influenzae type b, S. pneumoniae, and N. meningitidis. None of these three tests successfully detected group B streptococcal antigens in body fluids. Nonspecific reactions noted with the staphylococcal coagglutination test could be usually eliminated after premixing test specimens with soluble protein A.

Detection of bacterial antigens in body fluids has enabled rapid identification of the specific etiology of some infections, particularly meningitis (6). In cases of partially treated meningitis, when cultures have been sterile, demonstration of such antigens has been useful in establishing an etiology. Quantitative determination of circulating antigens may also be of prognostic significance (8). Counterimmunoelectrophoresis (CIE) (2, 4, 12, 18) and agglutination of antibody-coated latex particles (3, 14, 17, 21) have been used for detecting microbial antigens. CIE requires special equipment, takes at least 30 min (often longer) before the results can be read, and is inconvenient for testing individual specimens that arrive at irregular intervals. Although CIE is specific, its sensitivity is limited. Very early in the course of certain bacteremic infections, such as Haemophilus influenzae type b (HIB) epiglottitis (18) and experimental HIB infection in rats (10) the amount of circulating antigen may be too small to be detected by CIE. Also the quantity of antigen is proportionate to the concentration of bacteria in cerebrospinal fluid of children with HIB meningitis (8). Two recent reports indicate that the latex agglutination test (LAT) is more sensitive than CIE in detecting HIB antigen in body fluids of children (20) and of experimental animals (D.W. Scheifele, R. Daum, V. Syriopoulu, G. Siber, and A. Smith, Pediatr. Res. 11:505, 1977). Although LAT is more sensitive than CIE, false-positive reactions have been noted with LAT due to heat-labile interfering substances (14) and to the presence of rheumatoid factor or other cold-reactive immunoglobulin M (IgM) antibodies (16).

The staphylococcal coagglutination test (SCT) is another means of detecting bacterial antigens. Protein A-rich staphylococci bind to the Fc fragment of some subclasses of IgG leaving the Fab fragment free. In the presence of the homologous antigen, antibody-coated staphylococci agglutinate. SCT has been used for the identification and serotyping of some bacterial isolates (11, 15) and in demonstrating meningococcal antigen in cerebrospinal fluid (15). An earlier report from this laboratory described the usefulness of the SCT in the identification of HIB and in the detection of its soluble antigens in body fluids (19). SCT was simple to perform and was more sensitive than CIE in detecting HIB antigens. This report is an expansion of the use of SCT for detection of several bacterial antigens in body fluids and a comparison of SCT to LAT and CIE.

MATERIALS AND METHODS

Specimens. Cerebrospinal fluid (CSF) received in the bacteriology laboratory was tested, after appropriate cultures and Gram stain preparations were made, for the presence of bacterial antigens by SCT, CIE, and LAT. All CSF specimens were tested by SCT and CIE; however, not enough CSF was available to test all specimens by LAT. If a specimen could not be tested immediately, it was refrigerated and tested within 24 h. Some specimens were stored at −20°C and tested within 3 days. Serum specimens were obtained from the same patients with meningitis and from some bacteremic patients at the time of admis-
sion, or within a few days thereafter. Unconcentrated urine was obtained serially from a selected number of the same patients. Sera and urines were either tested immediately or stored at −20°C until tested. HIB specimens tested do not include ones reported in the earlier communication (19).

All tests were performed by one of the authors. CSF specimens were examined for presence of antigens without knowledge of results of cultures of Gram smear preparations. Many serum and urine samples were obtained, however, after a bacterial etiology had been known.

Antiserum. Antiserum against HIB was initially purchased from Hyland Division of Travenol Laboratories. Subsequently we raised anti-HIB antiserum in rabbits by the intravenous injection of Formalin-treated whole organisms, as described by Alexander et al. (1). By using antiserum prepared in our laboratory, we were able to detect 0.05 μg of HIB capsular polysaccharide per ml (obtained from John B. Robbins) by CIE. Hyland antiserum could not detect less than 0.2 μg of the capsular polysaccharide per ml. Pneumococcal omnisera was purchased from the Staten Serum-institut, Copenhagen, Denmark. Group B streptococcal antisera from two sources were used. Type-specific rabbit antisera were raised (22) in our laboratory. These antisera have been used in serotyping group B streptococcal isolates by the capillary precipitin method and by CIE. Strong reactions were noted by both methods, and serotype identification was in full concordance with serotyping of the same isolates at the Center for Disease Control, Atlanta. A pool of the type-specific antisera was used in the present study. Group B streptococcal grouping antisera was purchased also from Burroughs Wellcome Co. (lot K2727). Two pools of meningococcal antisera (pool 1, serogroups A, B, C, and D, lot K1948; pool 2, sero- groups X, Y, and Z, lot K1489) were also purchased from Burroughs Wellcome Co.

CIE. CIE was performed according to a standard method (7) as previously modified (5). When possible, each specimen was tested for antigens of HIB, S. pneumoniae, N. meningitidis, and group B streptococcus by using the above mentioned antiserum.

SCT. Stabilized staphylococcal suspensions were prepared as described in an earlier report from this laboratory (19). Antibody-coated staphylococcal suspensions using the various specific antisera were also prepared.

The control reagent consisted of staphylococci coated with a normal rabbit serum free of antibodies to HIB, S. pneumoniae or N. meningitidis. A drop of the clinical specimen was placed in a well of an agglutination test slide and an equal amount of the appropriate antibody-coated staphylococcal suspension was added. The specimen and suspension were mixed thor- oughly with a wooden toothpick. The agglutination reaction was read at the end of 2 min against a dark background with the help of a magnifying lens. The agglutination reaction was graded as 0 (negative) when the mixture remained a uniform suspension, as 1+ when only minimal granularity was evident, as 2+ when granularity was readily apparent but the mixture remained cloudy, as 3+ when clumps were noted, and as 4+ when coarse clumps of agglutination occurred. The test was considered positive when 2+ or stronger agglutination was noted with a single antibody-coated staphylococcal suspension, and no agglutination was noted with the control or with staphylococci coated with the other antibodies.

Some body fluids caused agglutination of the control and of all the antibody-coated staphylococcal suspensions. However, such nonspecific agglutinations were of low intensity (1+). In such instances the test was considered positive for a specific antigen when agglutination with a single antibody-coated suspension was 3+ or 4+. When agglutination with control and test suspensions were of equal or nearly equal intensity, the test was considered negative.

Soluble protein A and confirmatory coagglutination test. Soluble staphylococcal protein A was prepared by the method described by Forsgren and Sjöquist (9) with the omission of ethanol and trichlo- roacetic acid precipitation steps. The preparation contained 600 μg of protein A per ml. Some specimens from patients with HIB infections and some nonspecifically reacting clinical specimens were preincubated with protein A solution (by adding a drop of the protein A solution to a drop of the specimen) for 1 min before addition of two drops of the reagent staphylo- cocci (15).

LAT. The test reagents for LAT were prepared by coating latex particles (0.81 μm, Difco, Detroit, Mich.) with gamma globulin precipitated from the specific antiserum, according to the method of Whittle et al. (21). Latex particles coated with normal rabbit gamma globulin served as a control.

RESULTS

The detection of various bacterial antigens by the three methods in specimens obtained before or during the first 3 days of illness in patients with bacteriologically documented infections is shown in Table 1. HIB antigens were most readily detected in all body fluids by SCT. CIE was the least sensitive. Smaller numbers of speci- mens were available from patients with diseases due to the other organisms. In eight patients with pneumococcal infection, at least one body fluid had detectable antigen by SCT in four cases, by LAT in three cases and by CIE in only one case. SCT and LAT were each positive in two of six patients with meningococcal infection, but CIE was positive in only one case. Patients with pneumococcal and meningococcal infection are too few to allow statistical comparison to patients with HIB infections. None of the three methods was effective in identifying group B streptococcal antigens in body fluids.

The persistence of HIB antigens in CSF, serum, and urine was examined sequentially in some patients (Table 2). Before therapy was started, all tests were effective in detecting HIB antigens. SCT and LAT were more sensitive than CIE, however. After initiation of therapy,
antigens were less commonly detected in body fluids by all three tests, and CIE was the least sensitive.

We were unable to detect HIB antigen by CIE in CSF specimens obtained 10 or more days after treatment. LAT detected the antigen in one such specimen and SCT was positive in three instances. Antigen was detectable only by SCT in two CSF specimens obtained from a patient on days 14 and 20 of treatment but was not detectable on day 25. This patient developed obstructive hydrocephalus during the course of HIB meningitis.

In no instance was CIE positive when SCT or LAT was negative. One urine specimen obtained from a patient with HIB meningitis 3 days after hospitalization had a positive LAT but a negative SCT. In 11 specimens (4 CSF, 2 sera, 5 urines) the HIB antigen was detected by SCT but not by LAT.

Storing the specimens at 4°C for up to 72 h or at −20°C for up to 6 months had no effect on the test results. In retesting 68 CSF, 45 serum, and 30 urine specimens that had been stored, the results were the same before and after storage.

Nonspecific agglutinations with SCT were noted in 30 CSF specimens. A total of 16 of these were bloody. The other 14 nonspecifically reacting specimens were from 5 patients: 2 who had undergone recent surgery for brain tumors (one of whom also had a recent shunt infection caused by coagulase-negative staphylococci); an infant who had recent *Klebsiella pneumoniae* meningitis; a 2-year-old child with aseptic meningitis; and a newborn who had suffered an intraventricular hemorrhage. These blood-free CSF samples had elevated levels of protein (range 165 to 530 mg per 100 ml), except in the neonate (CSF protein 69 mg per 100 ml). A total of 12 of the 16 blood-stained specimens and 13 of the 14 blood-free specimens caused nonspecific agglutination with LAT also.

In an attempt to eliminate nonspecific reactions, soluble protein A was premixed with some nonspecifically reacting CSF specimens (Table 3). Protein A eliminated most of the nonspecific reactions in bloody CSF specimens and in ones with an elevated protein content; however, specific agglutination reactions were not affected.

To determine the lowest quantity of soluble protein A that would eliminate the nonspecific agglutination, serial 2-fold dilutions of a protein A solution were made and two nonspecifically reacting CSF specimens were premixed with each dilution before testing with the antibody-coated staphylococcal suspensions. Nonspecific agglutinations could be eliminated by using protein A concentrations of 75 and 37.5 μg/ml, respectively.

Nonspecific agglutinations were seen also with 18 of 63 serum specimens and 15 of 54 urine specimens tested. Preincubation with protein A eliminated the nonspecific reactions in all 12 serum specimens tested and in 11 of the 15 urine specimens.
SCT and LAT did not misidentify an antigen in any instance; however misidentification was noted once with CIE. In a CSF sample that subsequently grew *N. meningitidis*, no meningococcal antigen was detected by SCT, LAT, or CIE. A precipitin line between the CSF well and the well containing the pneumococcal omnisemum was noted by CIE.

Four sera positive for rheumatoid factor were tested to evaluate the extent to which rheumatoid factor might cause false-positive reactions. No agglutinations of the control or antibody-coated suspensions were noted in LAT or SCT.

**DISCUSSION**

The data presented indicate that SCT is a simple and sensitive test for detecting HIB antigens in body fluids. When clinical specimens are tested without concentration, SCT is at least as sensitive as LAT and is more sensitive than CIE in identifying HIB antigens both before and during the treatment of HIB infections. Whereas SCT less commonly detected pneumococcal (58%) and meningococcal (32%) antigens than it did HIB antigens (88%), SCT was at least as sensitive as LAT or CIE. The poor results in detecting meningococcal antigens may be because most of our meningococcal isolates were subsequently found to be nontypable.

Despite the use of antisera from two different sources, we were unable to detect group B streptococcal antigens in body fluids, even in CSF specimens yielding a heavy growth of the organism. The antisera were used adequate for serotyping and grouping; however, a more potent antiserum may have been necessary for detecting antigens in body fluids. Rhodes and Hall (J. Pediatr. 91:833, 1978) were able to detect group B streptococcal antigens by CIE in urines of 6 of 12 neonates, but only 1 of 10 sera tested was positive. The urines in this study were concentrated 20-fold, and this may have increased the number of positive reactions. In our study, body fluids were not concentrated.

Hemolyzed sera, bloody CSF, and some CSF specimens with high protein content caused non-specific agglutination reactions in both SCT and LAT. Olgen also observed such nonspecific agglutination in SCT (15). The exact cause of this nonspecific reaction is unknown but may be due to the very widespread prevalence of pneumococcal antibodies in human sera (13). Olgen and co-workers were able to eliminate the nonspecific agglutination by premixing CSF specimens with soluble protein A (15). We were also able to eliminate most of the nonspecific agglutinations in SCT by premixing CSF, serum or urine, with soluble protein A. Such premixing eliminated nonspecific reactions in LAT only infrequently. Protein A had no effect on the specific agglutination reaction of specimens containing bacterial antigens. Mixing with soluble protein A probably removes antibodies present, without affecting bacterial antigens.

Based on our experience with SCT we believe that it is a specific, simple, and fast test that is a useful addition to CIE and LAT for the detection of bacterial antigens in body fluids.

**ACKNOWLEDGMENTS**

This study was supported by a grant from the Matilda Wilson Fund, Detroit, Mich. The secretarial assistance of Noralee Cyplik is gratefully appreciated.

**LITERATURE CITED**


