Clinically Significant Cross-Reactions with Counterimmunoelectrophoresis between Pneumococcus Type 6 and *Haemophilus influenzae* Type b

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Biologically and clinically significant cross-reactions may occur between the immunochromatically similar capsule antigens of *Haemophilus influenzae* type b and *Streptococcus pneumoniae* type 6 by using counterimmunoelectrophoresis (CIE). In three cases of culture-proven *S. pneumoniae* type 6 disease, a false-positive *H. influenzae* type b CIE result was detected in a body fluid. Two of three cases were also positive when tested by CIE with pneumococcal omnisera. However, in one of the culture-proven cases of pneumococcal type 6 disease, the omnisera was negative and only the burro 132 *H. influenzae* type b antiserum demonstrated a positive precipitant reaction. In addition to the three case isolates, we evaluated nine blood or cerebrospinal fluid isolates of pneumococcus type 6, using a variety of both species- and type-specific antisera, by CIE. Two burro antiserums detected 12 of 12 (100%) of the *S. pneumoniae* type 6 strains. A rabbit *H. influenzae* type b antiserum did not detect any (0 of 12; 0%) of the *S. pneumoniae* type 6 strains evaluated. All (12 of 12; 100%) of the *S. pneumoniae* type 6 strains were detected with both rabbit omnisera (*S. pneumoniae*) and rabbit type 6 antiserum. Our data illustrate the importance of being aware of the immunologic cross-reactivity of the *H. influenzae* type b capsule with the pneumococcus type 6 capsule and the possibility of false-positive results with CIE when patient specimens are interpreted.

Counterimmunoelectrophoresis (CIE) is useful as a rapid means of detecting and identifying bacterial antigens in patient specimens of blood, urine, and cerebrospinal fluid (CSF). We report here the occurrence of immunologic cross-reactions among related antigens. Specifically, it has been documented that the capsular polysaccharide of *Haemophilus influenzae* type b is immunochromically similar to that of certain other antigens, including *Streptococcus pneumoniae* type 6 (9). We demonstrated that burro antiserum to *H. influenzae* type b cross-reacts with pneumococcus type 6 antigens with CIE. We report three cases in which patient specimens gave positive precipitin lines in CIE with both *H. influenzae* type b antiserum and omni (pneumococcal diagnostic antiserum). In all three cases, it was proven by culture that the patients were infected with pneumococcus type 6. These cases illustrate the importance of being aware of this cross-reaction and the possibility of false-positive results with CIE.

**CASE REPORTS**

Patient one was a 2½-year-old girl with a 6-day history of vomiting, fever, irritability, lethargy, and diarrhea. Therapy with amoxicillin was begun 3 days prior to admission. Meningeal irritation was noted on admission. Initial laboratory work-up revealed a CSF with 200 leukocytes per mm³, a protein level of 74 mg/dl and a glucose level of 123 mg/dl (glucose in serum was 233 mg/dl). A Gram stain of the CSF showed gram-positive diplococci. CIE on CSF was positive for pneumococcal antigen at 2 h, and at the 24-h reading it was also positive for the *H. influenzae* type b antigen. Therapy was initiated with chloramphenicol and ampicillin. Subsequently, the CSF culture grew *S. pneumoniae* type 6, which is susceptible to penicillin. Chloramphenicol therapy was discontinued, and the patient completed a course of therapy for pneumococcal meningitis.

Patient two, a 15-year-old female, had a 3-day history of headache, malaise, and nuchal rigidity prior to admission. On admission, analysis of the CSF showed 5,822 leukocytes per mm³, a protein level of 181 mg/dl, and a glucose level of 68 mg/dl (glucose in serum was 210 mg/dl). A Gram stain of CSF revealed leukocytes and gram-positive diplococci, and penicillin therapy was begun. Urine and CSF were both negative by CIE for pneumococcus, but the CSF was positive for *H. influenzae* type b. Therapy was then changed to ampicillin and chloramphenicol. CSF and blood cultures grew *S. pneumoniae*. Ampicillin and chloramphenicol therapy was discontinued, and penicillin therapy was begun. The pneumococcus was then typed and discovered to be type 6.

Patient three was a 17-month-old male with bronchopulmonary dysplasia, who was premature at birth and had a history of five previous hospital admissions. For 1 week prior to admission, the patient had rhinorrhea. On the morning of admission, the patient developed right-side tonic-clonic seizures, and had a fever of 103°F (39°C). Intravenous valium and phenobarbital were administered. A lumbar puncture revealed a cloudy fluid with 1,584 leukocytes per mm³, a protein level of 74 mg/dl, and a glucose level of 57 mg/dl (glucose in serum was 61 mg/dl). A Gram stain revealed gram-positive and occasional gram-negative diplococci. The CSF was tested by CIE and was positive for both *H. influenzae* type b and pneumococcus.

Based on the ambiguous nature of the Gram stain and the confusing results in CIE, treatment was initiated with chloramphenicol and ampicillin. At 48 h, however both the CSF...
and blood cultures grew only pneumococcus, so ampicillin and chloramphenicol therapy was discontinued, and therapy with penicillin was begun. The organism was typed by CIE and found to be type 6.

MATERIALS AND METHODS

Patient specimens included blood, CSF, and urine. The initial CIEs on patients one and three were done with CSF. Urine (concentrated 25 times with a Minicon B-15 filter) and CSF specimens from patient two were tested by CIE. Pneumococcus type 6 organisms isolated from patient specimens (blood or CSF) and all other isolates used in our experiments were typed by CIE at the time of isolation. All were frozen at −70°C in 1 ml of rabbit blood. All organisms were subcultured to sheep blood agar plates. The next day a brain heart infusion broth suspension was incubated for 4 h at 35°C, and this solution was tested against the antisera by CIE.

Burro 132 antiserum directed against *H. influenzae* type b was obtained from John B. Robbins, Bureau of Biologics, Food and Drug Administration. Burro D1 antiserum directed against *H. influenzae* type b was prepared in our laboratory and used as an additional source of *H. influenzae* type b antisera with a high titer. The rabbit antiserum to *H. influenzae* type b was obtained from Wellcome Reagents Limited, Beckenham, England. All diagnostic pneumococcal antisera (omni, pools, and types) were obtained from Statens Serum研究所, Copenhagen, Denmark. Preimmunized burro serum was taken from our stock. For the CIE procedure, glass slides (50 by 75 mm) were coated with 12 ml of 1% agarose in barbital buffer. Parallel rows of opposing sets of wells, 3 mm in diameter and 3 mm apart, were cut into the agar. Each well contained approximately 15 μl of fluid. A Hyland power source supplied the current (40 mA). Electrophoresis was carried out for 60 min. Precipitin lines were read immediately after electrophoresis and again after 18 h of refrigeration. A hand lens was used to view the lines.

RESULTS

In addition to the three cases cited above, we evaluated specimens from nine other patients with culture-proven *S. pneumoniae* type 6 disease. Of the 12 patients with *S. pneumoniae* type 6 disease, a body fluid from 6 patients was sent for CIE at the time of admission (three CSF, three concentrated urine). Two specimens were positive when tested against burro 132 *H. influenzae* type b antiserum and omni serum, and one specimen was positive with burro 132 *H. influenzae* type b antiserum and negative with omni serum. One specimen was positive with omni serum and negative with burro 132 *H. influenzae* type b antiserum. The fifth specimen was negative when tested against both burro 132 *H. influenzae* type b antiserum and omni serum, and the last specimen was tested with only burro 132 antiserum (omni serum was not tested) and was negative. Initially, therefore, three of six (50%) specimens from patients with documented *S. pneumoniae* 6 disease cross-reacted with burro 132 *H. influenzae* type b antiserum; one of six (14%) was positive with burro 132 *H. influenzae* type b antiserum only.

We evaluated a variety of antisera, both species and type-specific: rabbit omni serum (*S. pneumoniae*), burro 132 (*H. influenzae* type b), burro D1 (*H. influenzae* type b), rabbit *H. influenzae* type b, rabbit pneumococcal type 6, and normal (preimmunized) burro sera. These results are summarized in Table 1. The burro 132 (*H. influenzae* type b) antiserum and burro D1 (*H. influenzae* type b) detected 12 of 12 (100%) of the *S. pneumoniae* type 6 strains. The rabbit *H. influenzae* type b antiserum did not detect any of these 12 (0%) of the *S. pneumoniae* type 6 strains evaluated. All 12 of 12 (100%) of the *S. pneumoniae* type 6 strains were detected with both omni sera and rabbit type 6 antiserum.

Two *S. pneumoniae* type 23 and one type 19 were run similarly with the same five antisera. All were positive against rabbit omni sera but negative against burro 132, rabbit *H. influenzae* type b, pneumococcus type 6, and normal burro sera.

We also tested five strains of *H. influenzae* type b against *S. pneumoniae* type 6 and omni sera and found no cross-reactions.

DISCUSSION

This report documents three cases of pneumococcal type 6 disease associated with a biological false-positive *H. influenzae* type b CIE on a body fluid. In two of these cases, the pneumococcal omnisera also gave a positive result. However, in one of the culture-proven cases of pneumococcal type 6 disease, the omni sera gave a negative result, and only the burro 132 *H. influenzae* type b antiserum gave a positive result.

In our laboratory we have identified 127 isolates of *S. pneumoniae* from blood and CSF cultured between January 1979 and August 1982. Of these 127, 73 were typed with pneumococcal pools and types by CIE, and 11 of these 73 (15%) were type 6. A national incidence of *S. pneumoniae* type 6 disease is difficult to calculate because of the limited number of isolates being routinely typed. Since 1967, Austrian has accumulated data on 4,000 *S. pneumoniae* isolates (2). Of note is his ranking of the top 12 types or groups of *S. pneumoniae* that cause bacteremia in children under the age of 12. These types were ranked as follows: 14, 6, 23, 19, 18, 4, 9, 7, 1, 3, 8, and 15. In addition, he found that between 50 and 60% of bacteremia isolates in this age group will be of types or groups 6, 14, 19, and 23 (2).

Although we did not find a cross-reaction between rabbit *H. influenzae* type b antiserum and our *S. pneumoniae* type 6 organisms by CIE, this cross-reaction (between rabbit *H. influenzae* type b and *S. pneumoniae*) has been noted previously by the Quelling reaction (13, 15). Tunwall has reported that 3 of 10 (30%) of *S. pneumoniae* type 6 organisms gave a positive capsular swelling reaction with anti-*H. influenzae* type b rabbit serum (13). In 1979, Finch and Wilkinson tested one *S. pneumoniae* type 6 isolate
against four different sources of rabbit H. influenzae type b antisera using CIE and found no cross-reactions (4). We determined the titers of our burro 132 antiserum (H. influenzae type b) against the Wellcome rabbit H. influenzae type b antiserum using an experimental polyribosyl phosphate H. influenzae type b vaccine (Lederle Laboratories, Pearl River, N.Y.) as the antigen. The burro 132 H. influenzae type b antiserum detected 3 ng of polyribosyl phosphate per ml, while the rabbit H. influenzae type b antiserum detected a minimum of 50 ng/ml, showing at least a 10-fold greater sensitivity of the burro antisem. This may explain our inability to detect this cross-reaction using this rabbit antisem.

In 1943, Neter referred to “the presence of antibodies directed against the specific soluble substance of this microorganism” when trying to explain the basis of the cross-reaction between H. influenzae type b horse serum and S. pneumoniae type 6 (7). The pneumococcal type 6 organism has been shown to contain ribitol as the carbohydrate component of its polysaccharide capsule (8). The type b polysaccharide of H. influenzae is also reported to be composed of polyribosylribitol phosphate (10, 12, 14). Many other organisms, also containing ribitol, have been shown to cross-react with H. influenzae type b antiserum. S. pneumoniae types 15, 29, and 34 cross-reacted in precipitin and capsular swelling tests (6, 7, 15) with H. influenzae type b antiserum. Staphylococcus aureus, Bacillus pumilus, and Lactobacillus plantarum (1), in addition to Escherichia coli K100 and Streptococcus viridans, have been shown to give a precipitin halo when plated by the antiserum agar technique (3, 11) (agar containing H. influenzae type b antiserum). Staphylococcus aureus and Escherichia coli O75 were also shown to give a precipitin line when tested against rabbit H. influenzae type b antiserum by CIE (4). Because ribose is a common capsular polysaccharide, the potential exists for cross-reactions to be found with many more encapsulated bacteria with a high-titer H. influenzae type b antiserum. It should be noted that when latex particles coated with rabbit antibody to H. influenzae type b were used, one investigator found no cross-reactions using patient specimens (CSF, blood, urine) known to contain S. pneumoniae (5). It is not known whether any of the patients in that study had S. pneumoniae type 6 disease, because typing of the organisms was not studied.

In our three patients, knowledge of the immunologic cross-reactivity of the H. influenzae type b capsule with the pneumococcus type 6 capsule was important in interpreting CIE results. Biologically and clinically significant cross-reactions can occur when rapid diagnostic methods based on immunologic principles are used.

LITERATURE CITED