Nontypeable *Haemophilus influenzae* as a Cause of Spontaneous Bacterial Peritonitis

Daniel M. Musher,^{1,2,3*} Aran Cunningham Nichol,^{1,2} and Adriana M. Rueda¹

Medical Care Line (Infectious Disease Section), Michael E. DeBakey Veterans Affairs Medical Center, Houston, Texas,¹ and the Departments of Medicine² and Molecular Virology and Microbiology,³ Baylor College of Medicine, Houston, Texas 77030

Received 3 October 2005/Returned for modification 22 November 2005/Accepted 12 April 2006

***Haemophilus influenzae* rarely causes spontaneous bacterial peritonitis. We describe a typical case of spontaneous bacterial peritonitis in which the causative organism was identified as nontypeable *H. influenzae*, biotype III. Infection progressed despite the presence of adequate serum bactericidal antibody, probably due to the absence of complement in ascites fluid.**

CASE REPORT

A 54-year-old former intravenous drug user and alcoholic, known to have chronic hepatitis C infection, presented with 3 days of severe abdominal pain, chills, and fever. He had no symptoms of upper or lower respiratory tract disease. His temperature was 104°F. His skin showed jaundice with spider angiomas. The abdomen was tense and exquisitely tender, with peritoneal signs and a fluid wave. His white blood cell count was 8,200 (5% band forms); bilirubin, 2.5 mg/dl; serum glutamic oxalacetic transaminase, 64 units (normal, <45); and albumin, 2.5 mg/dl. His prothrombin and partial thromboplastin times were markedly elevated. Computed tomography showed no pulmonary infiltrates, a shrunken, nodular liver, and a large amount of ascitic fluid. The diagnosis was spontaneous bacterial peritonitis. Because of the abnormal clotting studies, a peritoneal tap was not done. Blood cultures revealed nontypeable *Haemophilus influenzae*, biotype III.

Bacteriological studies. The infecting organism grew on chocolate but not blood agar; the colonial appearance and morphology on microscopic examination of a gram-stained specimen were typical for *H. influenzae*. The isolate required X and V factors for growth. On biochemical testing, it was indole and ornithine decarboxylase negative and urease positive. The API NH strip, the 16S rRNA characterization, and traditional biochemicals (done in the Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA) confirmed the identification of *H. influenzae*, biotype III.

The bactericidal activities of acute- and convalescent-phase sera from the patient were tested against his own isolate, using methods that we have previously described (15). Briefly, the organism was cultivated overnight on chocolate agar at 37°C in an atmosphere containing 5% CO₂. Colonies were removed with a bacteriologic loop and suspended in sterile phosphate-buffered saline to a concentration of about 5×10^8 CFU per ml. Aliquots were added to brain heart infusion enriched with hemin and NAD to yield about 10^7 CFU per ml in a series of tubes that each contained one of the following: (i) 25% serum

from the patient on the day of admission (acute-phase serum), supplemented with 5% fresh rabbit serum to replete complement; (ii) 25% serum obtained from the patient 4 weeks after discharge from the hospital (convalescent-phase serum), supplemented with 5% fresh rabbit serum; (iii) 5% fresh (complement-rich) rabbit serum alone; (iv) 25% acute-phase and (v) 25% convalescent-phase patient serum after heating to 56°C for 20 min to destroy complement; (vi) 25% complement-rich human serum pooled from healthy young adults; or (vii) 25% heat-treated pooled human serum. Samples were incubated at 37°C. Aliquots were removed at hourly intervals for 4 h and diluted serially in phosphate-buffered saline. Ten-microliter aliquots were streaked on chocolate agar plates which were then incubated overnight in 5% CO₂. As shown in Fig. 1, the patient's serum from time of admission exhibited substantial bactericidal activity, and this activity was even greater in convalescence. Rabbit serum alone showed a slight bactericidal effect during the first 2 h of incubation and none thereafter. In contrast, serum from healthy adults was not bactericidal. As expected, heat-treated sera also exhibited no bactericidal activity.

Discussion. Spontaneous bacterial peritonitis results from bacterial seeding of preexisting ascitic fluid (5). In adults with cirrhosis, potential sources of the bacteria include the bloodstream, portal venous blood, and lymphatics that drain the bowel. Spontaneous bacterial peritonitis due to *Escherichia coli* is thought to result from an intestinal source, whereas that due to *Streptococcus pneumoniae* has generally been thought to be systemic (5). We (8) recently showed, however, that pneumococcal peritonitis often originates from an intestinal source or, in women, from a genital source.

Haemophilus influenzae is a rare cause of spontaneous bacterial peritonitis and has also been assumed to originate from a respiratory source. However, *H. influenzae* has been cultured from feces (13), jejunal fluid (10), and the genital tract (7, 17) of normal persons, as well as from 6% of appendices removed surgically from children with appendicitis (13). Although in most instances, sero- and/or biotyping was not reported, the appendiceal isolates were characterized: 80% were nontypeable, with biotypes II and III predominating. Of course, the

* Corresponding author. Mailing address: Infectious Disease Section, Room 4B-370, Veterans Affairs Medical Center, 2002 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 794-7384. Fax: (713) 794-7045. E-mail: daniel.musher@med.va.gov.

FIG. 1. Results of a representative experiment in which the patient's isolate was incubated in brain heart infusion supplemented with hemin and NAD containing one of the following: 25% serum pooled from healthy young adults; 25% pooled human serum after heat inactivation at 56°C for 20 min; 5% fresh-frozen rabbit serum (complement source); 25% serum obtained from the patient on the day of admission plus 5% rabbit serum to replete complement ("acute-phase serum"); and 25% serum obtained from the patient 1 month after recovery 5% rabbit serum ("convalescent-phase serum"). Tubes were incubated under 5% CO₂ at 37°C. Aliquots were removed hourly, and quantitative cultures were done. The experiment was done on three separate occasions, each time with similar results.

ultimate source might still be the nasopharynx; cirrhosis is associated with achlorhydria (2), which in turn permits colonization of the gut by upper respiratory flora (9).

Large series of cases of spontaneous bacterial peritonitis do not mention *Haemophilus* as a cause (5, 6, 23), but individual case reports have occasionally implicated this organism in spontaneous bacterial peritonitis (3, 19, 21) as well as in intra-abdominal abscess (1, 4, 12). Typing of the isolate was done in one reported case of spontaneous bacterial peritonitis and was said to be type b (19), a laboratory finding that may be in error (11, 22).

The nontypeable *H. influenzae* from our patient was not killed by complement-rich serum pooled from healthy young adults. In contrast, the patient's serum at admission exhibited bactericidal activity, presumably immunoglobulin G (IgG) mediated; an even greater degree of bactericidal activity was present in convalescence. We have reported similar bactericidal activity in acute-phase sera of patients with pneumonia due to nontypeable *H. influenzae* (16), with an increase in convalescence. We showed that, in pneumonia, IgA in bronchial secretions blocked the interaction of the bacteria with IgG (14). In the present case, progression of peritoneal infection despite the presence of antibody was more likely related to

low levels of complement in ascitic fluid (18, 20). The bacteria may spill into, but not survive very long in, the blood, nevertheless yielding positive blood cultures.

The assumption that the finding of *H. influenzae* in spontaneous bacterial peritonitis reflects a systemic, rather than an intestinal, origin (19) is probably unfounded. It seems reasonable to consider a gastrointestinal source, especially for a patient who has no evidence for upper or lower respiratory infection.

This work was funded in part by the Department of Veterans Affairs through the Merit Review Program.

Mary Jordan Hughes from the Centers for Disease Control and Prevention, Atlanta, GA, did the final confirmation of the identity of the isolate.

REFERENCES

1. Astagneau, P., F. W. Goldstein, S. Francoual, E. Baviera, M. Bartholon, and J. F. Acar. 1992. Appendicitis due to both *Streptococcus pneumoniae* and *Haemophilus influenzae*. *Eur. J. Clin. Microbiol. Infect. Dis.* 11:559–560.
2. Bauer, T. M., B. Steinbrückner, F. E. Brinkmann, A. K. Ditzgen, H. Schwacha, J. J. Aponte, K. Pelz, M. Kist, and H. E. Blum. 2001. Small intestinal bacterial overgrowth in patients with cirrhosis: prevalence and relation with spontaneous bacterial peritonitis. *Am. J. Gastroenterol.* 96:2962–2967.
3. Cereto, F., I. Gasser, G. Moreno, A. Smithson, A. Gonzalez, and J. Genesca. 2002. Spontaneous bacterial peritonitis caused by *Haemophilus influenzae*. *Rev. Esp. Enferm. Dig.* 94:40–41. (In Spanish.)
4. Christensen, J. J., E. Kirkegaard, and B. Korner. 1990. *Haemophilus* isolated from unusual anatomical sites. *Scand. J. Infect. Dis.* 22:437–444.
5. Conn, H. O., and J. M. Fessel. 1971. Spontaneous bacterial peritonitis in cirrhosis: variations on a theme. *Medicine (Baltimore)* 50:161–197.
6. Curry, N., R. W. McCallum, and P. H. Guth. 1974. Spontaneous peritonitis in cirrhotic ascites. A decade of experience. *Am. J. Dig. Dis.* 19:685–692.
7. Drouet, E. B., G. A. Denoyel, M. M. Boude, G. Boussant, and H. P. de Montclos. 1989. Distribution of *Haemophilus influenzae* and *Haemophilus parainfluenzae* biotypes isolated from the human genitourinary tract. *Eur. J. Clin. Microbiol. Infect. Dis.* 8:951–955.
8. Dugi, D. D., III, D. M. Musher, J. E. Clarridge III, and R. Kimbrough. 2001. Intraabdominal infection due to *Streptococcus pneumoniae*. *Medicine (Baltimore)* 80:236–244.
9. Husebye, E., V. Skar, T. Hoverstad, and K. Melby. 1992. Fasting hypochlorhydria with gram positive gastric flora is highly prevalent in healthy old people. *Gut* 33:1331–1337.
10. Justesen, T., O. H. Nielsen, K. Hjelt, and P. A. Krasilnikoff. 1984. Normal cultivable microflora in upper jejunal fluid in children without gastrointestinal disorders. *J. Pediatr. Gastroenterol. Nutr.* 3:683–686.
11. LaClair, L. L., M. L. C. Tondella, D. S. Beall, C. A. Noble, P. L. Raghunathan, N. E. Rosenstein, T. Popovic, and the Active Bacterial Core Surveillance Team Members. 2003. Identification of *Haemophilus influenzae* serotypes by standard slide agglutination serotyping and PCR-based capsule typing. *J. Clin. Microbiol.* 41:393–396.
12. McCarthy, L. G. 1981. *Haemophilus influenzae* associated with periappendiceal abscess. *Am. J. Gastroenterol.* 76:157–159.
13. Megraud, F., C. Bebear, H. Dabernat, and C. Delmas. 1988. *Haemophilus* species in the human gastrointestinal tract. *Eur. J. Clin. Microbiol. Infect. Dis.* 7:437–438.
14. Musher, D. M., A. Goree, R. E. Baughn, and H. H. Birdsall. 1984. Immunoglobulin A from bronchopulmonary secretions blocks bactericidal and opsonizing effects of antibody to nontypable *Haemophilus influenzae*. *Infect. Immun.* 45:36–40.
15. Musher, D. M., M. Hague-Park, R. E. Baughn, R. J. Wallace, Jr., and B. Cowley. 1983. Opsonizing and bactericidal effects of normal human serum on nontypable *Haemophilus influenzae*. *Infect. Immun.* 39:297–304.
16. Musher, D. M., K. R. Kubitschek, J. Crennan, and R. E. Baughn. 1983. Pneumonia and acute febrile tracheobronchitis due to *Haemophilus influenzae*. *Ann. Intern. Med.* 99:444–450.
17. Myhre, A. K., L. S. Bevanger, K. Berntzen, and D. Bratlid. 2002. Anogenital bacteriology in non-abused preschool children: a descriptive study of the aerobic genital flora and the isolation of anogenital *Gardnerella vaginalis*. *Acta Paediatr.* 91:885–891.
18. Runyon, B. A., R. L. Morrissey, J. C. Hoefs, and F. A. Wyle. 1985. Opsonic activity of human ascitic fluid: a potentially important protective mechanism against spontaneous bacterial peritonitis. *Hepatology* 5:634–637.
19. Salavert, M., A. Orero, L. Arguella, V. Olaso, C. Perez-Belles, and M. Gobernado. 1999. Recurrent spontaneous bacterial peritonitis due to encapsulated microorganisms (*Haemophilus influenzae* and *Streptococcus pneu-*

- moniae) in a patient with liver cirrhosis. *Gastroenterol. Hepatol.* **22**:30–31. (In Spanish.)
20. **Simberkoff, M. S., N. H. Moldover, and G. Weiss.** 1978. Bactericidal and opsonic activity of cirrhotic ascites and nonascitic peritoneal fluid. *J. Lab. Clin. Med.* **91**:831–839.
21. **Stephens, C. G., J. G. Meadows, T. M. Kerker, S. W. Markowitz, and R. M. Nisman.** 1979. Spontaneous peritonitis due to *Hemophilus influenzae* in an adult. *Gastroenterology* **77**:1088–1090.
22. **Wallace, R. J., Jr., D. M. Musher, E. J. Septimus, J. E. McGowan, Jr., F. J. Quinones, K. Wiss, P. H. Vance, and P. A. Trier.** 1981. *Haemophilus influenzae* infections in adults: characterization of strains by serotypes, biotypes, and beta-lactamase production. *J. Infect. Dis.* **144**:101–106.
23. **Weinstein, M. P., P. B. Iannini, C. W. Stratton, and T. C. Eickhoff.** 1978. Spontaneous bacterial peritonitis. A review of 28 cases with emphasis on improved survival and factors influencing prognosis. *Am. J. Med.* **64**:592–598.