Isolation of *Mycoplasma bovis* from a Patient with Systemic Illness

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Received for publication 12 March 1979

Mycoplasma bovis was cultured from the sputum of a patient with lobar pneumonia, psychosis, and probable myocarditis, nephritis, and hemolytic anemia. Although we cannot be certain that this species of mycoplasma was the etiological agent of the patient's acute illness, this case report is of interest because, to the best of our knowledge, it represents the first isolation of *M. bovis* from a human source.

Mycoplasma bovis is an organism that has been associated with bovine mastitis and with bovine infections of the joints and the respiratory tract (1, 4, 13, 14). To the best of our knowledge *M. bovis* has not previously been associated with human disease. In this report we describe a patient who had severe pneumonia with associated psychosis and probable myocarditis, nephritis, and hemolytic anemia. We isolated a strain of *M. bovis* from the patient's sputum but were unable to detect other etiological agents. We believe this to be the first instance in which *M. bovis* has been isolated from a human source.

(A preliminary report was presented at the First Conference of the International Organization for Mycoplasmaology [IOM], 15-18 September 1976, Glasgow, Scotland.)

MATERIALS AND METHODS

Case report. A 34-year-old white female was admitted to the Lynn Hospital because of fever and bronchopneumonia. Approximately 3 weeks prior to admission she had used cow manure as fertilizer while planting trees, but she denied direct contact with cattle. Five days before admission she noted the sudden occurrence of severe headaches, with marked photophobia, retrobulbar pain, and associated dizziness. Eight hours later she began to note fever and shaking chills. Her temperature was 104°F (40°C). When seen by her physician the following day, her temperature was 102.2°F (39°C), her heart rate at rest was 140, and her blood pressure was normal. She was given symptomatic treatment but did not improve. The following day she began to note visual and auditory hallucinations. Two days before admission her chest X-ray revealed right lower lobe bronchopneumonia, and she was started on oral erythromycin. However, because

she failed to improve, she was admitted to the hospital and the antibiotic was discontinued.

On admission, the patient was noted to be acutely ill. Her physical examination was within normal limits except that her temperature was 99.8°F (37.7°C), and coarse rales and rhonchi were noted over her right posterior lung base. Her cardiac rate was 120 in the supine position and increased to 160 on sitting up without postural change in blood pressure. Except for intermittent hallucinations, the patient's mental status and neurological examination were unremarkable. Admission laboratory studies included a hemoglobin (14.7 g/100 ml), hematocrit (44%), and leukocyte count and differential that were within normal limits. Urinalysis revealed the following abnormal findings: 3+ proteinuria, 2 to 4 erythrocytes, and 20 to 25 leukocytes per high-powered field with many granular and leukocyte casts. There were no erythrocyte casts, and routine urine culture was negative. A cold agglutinin titer was 1:2. The patient's chest X-ray revealed a dense infiltrate in the right lower lobe without evidence of pleural effusion. An electrocardiogram was unremarkable except for the presence of sinus tachycardia. An electroencephalogram was within normal limits. Sputum culture revealed no bacterial pathogens.

During hospitalization the patient remained febrile in the range of 100°F (27.8°C) to 101.5°F (38.6°C) for 14 days. A cold agglutinin titer repeated 2 weeks after admission was positive at 1:960. A serum thyroxine level was 6.8 mg/100 ml. Blood urea nitrogen and creatinine remained normal throughout her hospitalization. Creatinine clearance was 84 ml/min. LE (lupus erythematosus) preparation and antinuclear antibody tests were negative. She received tetracycline from days 7 through 13 of hospitalization. During this time, her pulse rate gradually returned to normal. Her hematocrit fell to 33% and her hemoglobin to 10.6 g/100 ml with a reticulocyte count of 2.4%. Serial chest X-rays revealed the persistence of dense right lower lobe infiltrate, which began to show slight clearing at the time of her discharge, 17 days after hospitalization.

After discharge from the hospital, the patient slowly improved. Her hematocrit and hemoglobin returned to normal. Serial chest X-rays revealed clearing of the infiltrate over a 1-month period. The patient's cold agglutinin titer reached a peak of 1:1,280 11 days after discharge from the hospital. One week later it had fallen to a level of 1:320.

Special studies. The presence of complement-fixing antibodies to *Mycoplasma pneumoniae*, rickettsia, psittacosis-lymphogranuloma venereum, salmonella, tularemia, brucellosis, and numerous respiratory viruses, as well as mumps and herpes simplex, was determined by standard methodology in the Virus Serology Laboratory of the Massachusetts Department of Public Health.

Sputum for mycoplasma isolation was plated on Hayflick medium (6), brucella agar with 5% horse blood (GIBCO Diagnostics, Madison, Wis.), and soft horse serum agar medium (8). Duplicate plates were incubated aerobically and anaerobically by the Fortner method (8). Plates were examined for the presence of mycoplasma with a magnifying hand lens and microscopically with the stained agar technique of Dienes (8).

For identification of the species of mycoplasma isolated by the above methods, the strain was cloned three times and a growth inhibition test was performed according to the method of Clyde (2) on each clone with antisera to the following organisms: *M. pneumoniae*, *M. hominis*, *M. fermentans*, *M. orale*, *M. buccale*, *M. salivarium*, and *Acholeplasma laidlawii*. These tests were performed in triplicate using hyperimmune sera prepared in our laboratory, sera received from the Bureau of Standards of the National Institutes of Health, and sera from Microbiological Associates, Inc., Bethesda, Md. The strain was studied further using the epifluorescence antibody test with fluorescein-conjugated globulins directed against a battery of 41 mycoplasma species or serogroups of human and animal origin (3). Growth inhibition tests were performed with hyperimmune sera to *M. bovis*, received from O. E. Stalheim of the National Animal Disease Center, Ames, Iowa (13), and to "Donetta," the type strain of *M. bovis*, received from M. E. Tourtellotte of the University of Connecticut, Storrs (1, 4).

RESULTS

Serological tests for rickettsia, psittacosis-lymphogranuloma venereum, salmonella, tularemia, brucellosis, and numerous respiratory viruses (including influenza A and B, adenovirus, respiratory syncytial virus, and parainfluenza 1, 2, and 3), as well as mumps and herpes simplex, were entirely negative. However, an early convalescent-phase serum revealed complement-fixing antibody to *M. pneumoniae* in a titer of 1:1,024. Eight months after discharge the patient's complement-fixing titer to *M. pneumoniae* was 1:64.

Sputum was cultured for mycoplasma by utilizing the methods described above. Mycoplasma colonies were first located at 96 h on

Hayflick medium (anaerobic) and only in the area of the inoculum. Within several days, mycoplasma colonies were also detected on aerobic and anaerobic horse blood agar and soft horse serum media. Slight acid production was noted in glucose-containing broth. Arginine was not hydrolyzed. The mycoplasma colonies produced "film and spots" on agar medium.

The colonies isolated were cloned three times, and growth inhibition tests were performed with antisera to *M. pneumoniae*, *M. hominis*, *M. fermentans*, *M. orale*, *M. buccale*, *M. salivarium*, and *A. laidlawii*. No inhibition of growth was observed with any of the antisera. No other strain of mycoplasma was detected after 6 weeks of incubation of the patient's cultures.

The mycoplasma strain isolated from this patient (called "Lynn") was studied further using the epi-immunofluorescence antibody test. Cultures of the Lynn strain growing on agar were stained with 1:20 dilutions of each of 41 conjugates directed against a battery of mycoplasma species or serogroups of human and animal origin. The homologous titers of the conjugates ranged from 1:320 to 1:2,560. A specific staining reaction was obtained only with one of the conjugates directed against strain HRC-213 isolated from bovine lung, presently classified as *M. bovis*. This conjugate was titrated against the two strains, and the fluorescence extinction titer was 1:320 for both. The Lynn strain and HRC-213 were thus indistinguishable by immunofluorescence. Growth inhibition tests were performed with hyperimmune serum to *M. bovis* and to Donetta, the type strain of *M. bovis*. In each test, there was a clear-cut zone of inhibition surrounding the disk containing type-specific antisera. All controls were negative.

Attempts were made to detect antibody to the bovine strain in the patient's serum. Growth inhibition, metabolic inhibition, and complement fixation tests using the patient's own mycoplasma strain were negative. Similarly, indirect immunofluorescence with anti-human globulin conjugate was also negative. However, since we did not have a known positive human control serum specimen, the negative results cannot be considered entirely conclusive.

DISCUSSION

To the best of our knowledge, this represents the first isolation of *M. bovis* from a human patient. It seems clear that this organism was isolated from the patient's sputum and was not a contaminant since we have not used bovine serum or any other bovine products in our laboratory and have not previously or subsequently isolated a bovine strain of mycoplasma.

It is more difficult to assess the role of this organism in the patient's illness. The illness itself is entirely consistent with disease caused by mycoplasma. The patient had lobar pneumonia without pleural effusion, which is a common manifestation of human disease caused by *M. pneumoniae* (5). Her transient psychosis was similar to the psychotic episodes that have been considered to be central nervous system manifestations of human mycoplasma infections (7). In addition, her persistent tachycardia without other obvious cause suggested that she likely had a mild myocarditis, a complication that has been described previously in mycoplasma infection (12). Finally, her initial urinalysis suggested a mild nephritis, and the fall in hematocrit and hemoglobin during the course of her hospitalization raised the possibility of hemolysis due to the cold agglutinins demonstrated in her serum. Thus, she had a severe infection with multisystem involvement (10). Her symptoms resolved with tetracycline therapy and she was left without permanent residual effects. Her entire clinical course was consistent with mycoplasma infection, and the rise and fall in titers of serum cold agglutinins add further support to this concept.

The isolation of *M. bovis* from the patient's sputum (in the absence of *M. pneumoniae* or other bacterial pathogens) raises several interesting possibilities. One is that *M. bovis* played a synergistic role in a pulmonary infection caused by a second pathogen, such as *M. pneumoniae*. Although we feel that this could be an explanation for the patient's illness, the failure to demonstrate serum antibodies directed against *M. bovis* makes it impossible to prove that it played a pathogenetic role in this case. The elevated titer of complement-fixing antibodies to *M. pneumoniae* (which decreased by 16-fold 8 months later) suggests strongly that the patient was infected with *M. pneumoniae* which could not be isolated from her sputum for technical reasons. If this were the case, the *M. bovis* might only have been a saprophytic colonizer of her upper respiratory tract. We have no evidence to suggest that the antibodies directed against *M. pneumoniae* in this patient represented cross-reacting antibodies elicited by a true infection with *M. bovis*.

Although the mycoplasma species have for many years been considered to be host specific, cross-infectivity of certain strains between human and animal hosts has been noted (9). The isolation of *M. bovis* in this case is of interest since heretofore it has not been isolated from

other than bovine sources. Investigations concerning the survival of *M. bovis* have shown that the organism is stable in milk, in straw, and in manure (11). Except for an exposure to cow dung 3 weeks before her illness, there was no history suggesting that the patient had any contact with cattle. Thus the exact source from which the patient acquired this organism will remain an enigma.

LITERATURE CITED

1. Askaa, G., and H. Erno. 1976. Elevation of *Mycoplasma agalactiae* subsp. *bovis* to species rank: *Mycoplasma bovis* (Hale et al.) comb. nov. *Int. J. Syst. Bacteriol.* **26**: 323-325.
2. Clyde, W. A. 1964. Mycoplasma species identification based upon growth inhibition by specific antisera. *J. Immunol.* **92**:958-965.
3. DelGiudice, R. A., N. F. Robillard, and T. R. Carski. 1967. Immunofluorescence identification of *Mycoplasma* on agar by use of incident illumination. *J. Bacteriol.* **93**:1205-1209.
4. Edward, D. G. ff., and E. A. Freundt. 1973. Type strains of species of the order Mycoplasmatales, including designation of neotypes for *Mycoplasma mycoides* subsp. *mycoides*, *Mycoplasma agalactiae* subsp. *agalactiae*, and *Mycoplasma arthritidis*. *Int. J. Syst. Bacteriol.* **23**: 55-61.
5. Harwick, H. J., G. M. Kalmanson, and L. B. Guze. 1972. Human diseases associated with mycoplasmas—with an appendix on simple culture techniques. *Calif. Med.* **116**:1-17.
6. Hayflick, L. 1965. Cell cultures and mycoplasmas. *Texas Rep. Biol. Med.* **23**(Suppl. 1):285-303.
7. Hodges, G. R., R. J. Fass, and S. Saslaw. 1972. Central nervous system disease associated with *Mycoplasma pneumoniae* infection. *Arch. Intern. Med.* **130**:277-282.
8. Madoff, S., and W. N. Pachas. 1970. Mycoplasma and the L-forms of bacteria, p. 195-217. In C. D. Graber (ed.), *Rapid diagnostic methods in medical microbiology*. The Williams and Wilkins Co., Baltimore.
9. Morton, H. E. 1970. Mycoplasma from man with undetermined specific relationships to their human host, p. 147-171. In J. T. Sharp (ed.), *The role of mycoplasma and the L-forms of bacteria in disease*. Charles C Thomas Publisher, Springfield, Ill.
10. Murray, H. W., H. Masur, L. B. Senterfit, and R. B. Roberts. 1975. The protean manifestations of *Mycoplasma pneumoniae* infection in adults. *Am. J. Med.* **58**:229-242.
11. Rinaldi, A., G. Redaelli, S. Nani, G. Ruffo, and A. Socci. 1972. Some observations on the epizootology of mycoplasmic bovine mastitis, p. 552-558. In *Proceedings of the World Association for Buiatrics Congress*. London.
12. Sands, M. J., Jr., J. E. Staz, W. E. Turner, Jr., and L. A. Soloff. 1977. Pericarditis and perimyocarditis associated with active *Mycoplasma pneumoniae* infection. *Ann. Intern. Med.* **86**:544-548.
13. Stalheim, O. H. V., and S. S. Stone. 1975. Isolation and identification of *Mycoplasma agalactiae* subsp. *bovis* from arthritic cattle in Iowa and Nebraska. *J. Clin. Microbiol.* **2**:169-172.
14. Tully, J. G. 1978. Biology of the Mycoplasmas, p. 1-33. In G. J. McGarrity, D. G. Murphy, and W. W. Nichols (ed.), *Mycoplasma infection of cell cultures*. Plenum Press, New York.