JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2001, p. 4568–4570 Vol. 39, No. 12
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.12.4568–4570.2001

Recurrent Soft Tissue Abscesses Caused by *Legionella cincinnatiensis*

JACQUES G. H. GUBLER,[1]* MIRJAM SCHORR,[1] V. GAIA,[2] R. ZBINDEN,[3] AND M. ALTWEGG[3]

Department of Medicine, Stadtspital Triemli, CH-8063 Zürich,[1] Swiss National Center for Legionella, Istituto Cantonale Batteriosierologico, CH-6904 Lugano,[2] and Institute for Medical Microbiology, University of Zürich, CH-8028 Zürich,[3] Switzerland

Received 13 June 2001/Returned for modification 18 July 2001/Accepted 5 September 2001

Recurrent soft tissue abscesses of the jaw, wrist, and arm developed in a 73-year-old housewife with nephrotic syndrome and immunoglobulin A(κ) gammopathy of unknown etiology. Conventional cultures remained negative, despite visible gram-negative rods on microscopy. Broad-spectrum PCR revealed *Legionella cincinnatiensis*, which was confirmed by isolation of the organism on special *Legionella* medium. Infections due to *Legionella* species outside the lungs are rare. *L. cincinnatiensis* has been implicated in only four cases of clinical infection; these involved the lungs in three patients and the central nervous system in one patient. We conclude that broad-spectrum PCR can be a valuable tool for the evaluation of culture-negative infections with a high probability of bacterial origin and that *Legionella* might be an underdiagnosed cause of pyogenic soft tissue infection.

Infections due to *Legionella* species have been described in numerous outbreaks and case reports ever since the first isolation of the organism following an outbreak at a convention of veterans in Philadelphia, Pa., in 1976 (9). Various *Legionella* species cause infections in persons exposed to common water-related sources and have been implicated in community and nosocomial outbreaks (5, 6, 8, 9, 12, 17–19). Patients with *Legionella* infections, especially immunocompromised patients, have a poor prognosis; however, *Legionella* infections only rarely affect organs outside of the lung. *Legionella cincinnatiensis*, first isolated from a dialysis patient with pneumonia in 1988 (25), has, until now, been implicated in clinical infections in only four patients, including three immunocompromised patients with pneumonia (14) and one patient with fatal encephalomyelitis following Pontiac fever (23). We report here on the first case of recurrent soft tissue abscesses caused by *L. cincinnatiensis*, occurring in a patient with only presumed mild immunosuppression due to nephritis and immunoglobulin A (IgA) gammopathy.

Case report. The patient was a 73-year-old housewife with no relevant medical history aside from treated arterial hypertension. In April 1999, a swelling on her left jaw disappeared spontaneously within 2 weeks. In June 1999, a large right cervical abscess was incised and drained. Gram-negative rods were seen on microscopy, but conventional aerobic and anaerobic cultures remained negative. Histology of the abscess wall showed nonspecific necrotizing granulocytic inflammation by hematoxylin-eosin staining. The patient was treated with oral amoxicillin-clavulanic acid at 1 g twice a day for 10 days. The wound healed over 3 weeks. Hematuria, proteinuria of 2,400 mg/24 h, and mild anemia (hemoglobin concentration, 10 g/dl) were noted at the time, with a normal renal function (serum creatinine level, 95 μmol/liter). Further evaluation revealed a gammopathy of undetermined etiology with an IgA(κ) paraprotein. One month later, her family physician excised an abscess from her left wrist; no samples for culture were taken. In November 1999, an abscess measuring 8 by 12 cm developed on her left arm, with an axillary lymph node measuring 5 by 5 cm. Puncture of the abscess yielded purulent fluid, with numerous gram-negative rods seen on microscopy. Again, conventional cultures and a search for mycobacteria by acid-fast staining and culture on solid and liquid media (BACTEC 460; Becton Dickinson) showed no growth. The fluid was examined by broad-spectrum PCR, and a positive result for bacterial DNA was obtained. Sequence analysis revealed *L. cincinnatiensis*. A large subfascial abscess extending to the humerus was surgically debrided; the axillary lymph node was not excised. Following retrieval of positive results by broad-spectrum PCR with the clinical sample from the initial puncture, cultures of the abscess were set up for conventional microorganisms as well as for *Legionella*. An organism later identified as *L. cincinnatiensis* was isolated. The patient was treated orally with clarithromycin (500 mg twice per day) combined with rifampin (600 mg per day) for 6 weeks. Two weeks after the cessation of therapy, a recurrent abscess at the same site had to be drained, and *L. cincinnatiensis* was again recovered from cultures of the abscess. The same antibiotic treatment was given for 8 weeks, with complete healing of the wound, disappearance of the axillary lymph node, and no recurrence of abscesses at a follow-up visit 13 months later. The patient did not report any travel outside Switzerland and denied unusual exposures through hobbies or daily activities.

Microbiology. The specimens were centrifuged at 1,711 × *g* for 15 min. Conventional cultures were set up on 5% sheep blood Columbia agar and on chocolate agar; and the cultures were incubated in 5% CO_2 at 35°C for 48 h, in thioglycolate broth at 30°C for 5 days, as well as on Schaedler's and Columbia-colistin nalidixic acid agar anaerobically at 35°C for 48 h. Following the positive result by broad-spectrum PCR, culture on special *Legionella* medium (buffered charcoal yeast agar

* Corresponding author. Mailing address: Department of Medicine, Stadtspital Triemli, CH-8063 Zürich, Switzerland. Phone: 41-1-466 11 11. Fax: 41-1-466 26 02. E-mail: jacques.gubler@triemli.stzh.ch.

[CYA; Difco] prepared in-house) was included. After 48 h, grayish round colonies with an irregular internal structure were seen on CYA. Gram staining showed thin gram-negative rods. For identification, subcultures were grown on CYA for 48 h at 37°C, and the cellular fatty acids were analyzed with the MIDI system (Microbial ID, Inc., Newark, Del.) as described earlier (7).

The main composition of fatty acids was as follows: $C_{i14:0}$, 11.3%; $C_{ai\ 15:0}$, 17%; $C_{15:1\omega6c}$, 5.9%; $C_{15:0}$, 2.8%; $C_{i16:0}$, 22%; $C_{16:1\omega7c}$, 21.5%; $C_{16:0}$, 6.5%; $C_{ai17:0}$, 3.4%; and $C_{cyc17:0}$, 5.5%. This fatty acid composition was very similar to that described previously for *L. cincinnatiensis* (7), with differences only in $C_{15:1\omega6c}$ (2.5% ± 0.2%) and $C_{cyc17:0}$ (11.0% ± 2.5%). *L. cincinnatiensis* is not included in the MIDI system database.

Susceptibility testing was performed by the E-test as described by the manufacturer (E-test; AB Biodisk) on buffered charcoal yeast extract (BCYE) medium (Oxoid). Several colonies were taken from a plate after 48 h of growth, were suspended in 0.85% NaCl to a turbidity corresponding to that of a 0.5 McFarland standard (10^8 CFU/ml), and plated onto BCYE medium. E-test antibiotic strips were placed onto plates, incubated at 35°C, and read after 48 h.

Susceptibility testing revealed a ciprofloxacin MIC of 0.032 mg/liter, an erythromycin MIC of 0.38 mg/ml, and a rifampin MIC of 1 mg/ml.

Broad-spectrum PCR for the detection of bacterial ribosomal DNA (rDNA) was done as described previously (10) with primers BAK-11w and PC3mod for primary amplification, resulting in a fragment of approximately 800 bp. This fragment was purified by horizontal polyacrylamide gel electrophoresis (Clean Gel 365; Amersham Pharmacia Dübendorf, Switzerland). After staining with silver, excision of the band was followed by reamplification with primers BAK-11w and BAK-533r.

Sequencing was done with fluorescence-labeled prime BAK-11w and the Autoload Solid Sequencing kit (Amersham Pharmacia), followed by analysis of the fragment on an automatic sequencer (ALF-Express; Amersham Pharmacia). The results revealed a complete match with the sequence of *L. cincinnatiensis* in the GenBank database (accession number X73407) at 263 nucleotides.

Total 16S rDNA sequencing with the ABI PRISM 310 Genetic Analyzer (AB Applied Biosystems) with the primers described previously (13) was also applied twice directly with the isolates cultured at the times of the two different surgical interventions. A BLAST program search for comparison of the 1,398-bp sequence obtained to the sequences stored in GenBank revealed homologies of 99.2, 98.5, 98.3, and 97.4% to *L. cincinnatiensis* (GenBank accession number Z49721), *Legionella sainthelensi* (GenBank accession number Z49734), *Legionella santicrucis* (GenBank accession number Z49735), and *Legionella bozemanii* (GenBank accession number Z49719), respectively. These values, in combination with the fatty acid composition data, confirm that the organism isolated indeed represents *L. cincinnatiensis.*

Both a direct immunofluorescence test for *Legionella pneumophila* done with a purulent secretion from the patient and a test for *Legionella* serotype 1 antigen done with the patient's urine were negative.

Discussion. We describe a patient with recurrent soft tissue abscesses in whom, after numerous negative conventional cultures, the search for bacterial DNA by broad-spectrum PCR finally led to the isolation of *L. cincinnatiensis*.

Legionella species, well described as agents of waterborne and nosocomial outbreaks of respiratory and systemic infections, especially among immunocompromised patients, have only rarely been implicated in extrapulmonary infections such as wound infections (3, 15, 20), endocarditis (22, 26), myositis (28), pericarditis (21), and cutaneous or perirectal abscesses (1, 2, 16, 27). In our patient, although no other infections have occurred, the presence of a paraprotein and of significant proteinuria might have led to immunosuppression sufficient to make the patient susceptible to infection with this unusal organism. Failure of primary antibiotic therapy, despite in vitro susceptibility, was ascribed to insufficient surgical drainage, since further therapy with the same agents was successful. We have not been able to elucidate for our patient any special source of exposure that could have led to her infection.

We conclude that *Legionella* species may be an underdiagnosed cause of extrapulmonary infections because they remain undetected unless special media are used. As in previous cases in our experience (4, 10, 11, 24), broad-spectrum PCR has been a valuable tool for the direction of investigations.

We acknowledge the help of Richard Hanselman, who cared for the patient.

ADDENDUM

In June 2001, a new large lymph node was excised from the patient's groin. Histology and further workup showed a disseminated diffuse large B-cell lymphoma. Following the second course of chemotherapy, the patient developed meningitis due to *Cryptococcus neoformans*. No recurrence of infection with *Legionella* was observed. The development of a high-grade malignant lymphoma might be due to transformation of a previously undiagnosed low-grade lymphoma. This would explain the gammopathy and therefore reflect some immunosuppression.

REFERENCES

1. **Ampel, N. M., F. L. Ruben, and C. W. Norden.** 1985. Cutaneous abscess caused by *Legionella micdadei* in an immunosuppressed patient. Ann. Intern. Med. **102:**630–632.
2. **Arnow, P. M., E. J. Boyko, and E. L. Friedman.** 1983. Perirectal abscess caused by *Legionella pneumophila* and mixed anaerobic bacteria. Ann. Intern. Med. **98:**184–185.
3. **Brabender, W., D. R. Hinthorn, M. Asher, N. J. Lindsey, and C. Liu.** 1983. *Legionella pneumophila* wound infection. JAMA **250:**3091–3092.
4. **Brunner, S., P. Frey-Rindova, M. Altwegg, and R. Zbinden.** 2000. Retroperitoneal abscess and bacteremia due to *Mycoplasma hominis* in a polytraumatized man. Infection **28:**46–48.
5. **Carratala, J., F. Gudiol, R. Pallares, J. Dorca, R. Verdaguer, J. Ariza, and F. Manresa.** 1994. Risk factors for nosocomial *Legionella pneumophila* pneumonia. Am. J. Respir. Crit. Care Med. **149:**625–629.
6. **Darelid, J., L. Bengtsson, B. Gastrin, H. Hallander, S. Lofgren, B. E. Malmvall, A. M. Olinder-Nielsen, and A. C. Thelin.** 1994. An outbreak of Legionnaires' disease in a Swedish hospital. Scand. J. Infect. Dis. **26:**417–425.
7. **Diogo, A., A. Verissimo, M. F. Nobre, and M. S. da Costa.** 1999. Usefulness of fatty acid composition for differentiation of *Legionella* species. J. Clin. Microbiol. **37:**2248–2254.
8. **Fiore, A. E., J. P. Nuorti, O. S. Levine, A. Marx, A. C. Weltman, S. Yeager, R. F. Benson, J. Pruckler, P. H. Edelstein, P. Greer, S. R. Zaki, B. S. Fields, and J. C. Butler.** 1998. Epidemic Legionnaires' disease two decades later: old sources, new diagnostic methods. Clin. Infect. Dis. **26:**426–433.
9. **Fraser, D. W., T. R. Tsai, W. Orenstein, W. E. Parkin, H. J. Beecham, R. G. Sharrar, J. Harris, G. F. Mallison, S. M. Martin, J. E. McDade, C. C.**

Shepard, and P. S. Brachman. 1977. Legionnaires' disease: description of an epidemic of pneumonia. N. Engl. J. Med. **297:**1189–1197.
10. **Goldenberger, D., A. Künzli, P. Vogt, R. Zbinden, and M. Altwegg.** 1997. Molecular diagnosis of bacterial endocarditis by broad-range PCR amplification and direct sequencing. J. Clin. Microbiol. **35:**2733–2739.
11. **Gubler, J. G., M. Kuster, F. Dutly, F. Bannwart, M. Krause, H. P. Vogelin, G. Garzoli, and M. Altwegg.** 1999. Whipple endocarditis without overt gastrointestinal disease: report of four cases. Ann. Intern. Med. **131:**112–116.
12. **Guiguet, M., J. Pierre, P. Brun, G. Berthelot, S. Gottot, C. Gibert, and A. J. Valleron.** 1987. Epidemiological survey of a major outbreak of nosocomial legionellosis. Int. J. Epidemiol. **16:**466–471.
13. **Jalava, J., P. Kotilainen, S. Nikkari, M. Skurnik, E. Vanttinen, O. P. Lehtonen, E. Eerola, and P. Toivanen.** 1995. Use of the polymerase chain reaction and DNA sequencing for detection of *Bartonella quintana* in the aortic valve of a patient with culture-negative infective endocarditis. Clin. Infect. Dis. **21:**891–896.
14. **Jernigan, D. B., L. I. Sanders, K. B. Waites, E. S. Brookings, R. F. Benson, and P. G. Pappas.** 1994. Pulmonary infection due to *Legionella cincinnatiensis* in renal transplant recipients: two cases and implications for laboratory diagnosis. Clin. Infect. Dis. **18:**385–389.
15. **Kalweit, W. H., W. C. Winn, Jr., T. A. Rocco, Jr., and J. C. Girod.** 1982. Hemodialysis fistula infections caused by *Legionella pneumophila*. Ann. Intern. Med. **96:**173–175.
16. **Kilborn, J. A., L. A. Manz, M. O'Brien, M. C. Douglass, H. M. Horst, W. Kupin, and E. J. Fisher.** 1992. Necrotizing cellulitis caused by *Legionella micdadei*. Am. J. Med. **92:**104–106.
17. **Kirby, B. D., and A. A. Harris.** 1987. Nosocomial Legionnaires' disease. Semin. Respir. Infect. **2:**255–261.
18. **Knirsch, C. A., K. Jakob, D. Schoonmaker, J. A. Kiehlbauch, S. J. Wong, P. Della-Latta, S. Whittier, M. Layton, and B. Scully.** 2000. An outbreak of *Legionella micdadei* pneumonia in transplant patients: evaluation, molecular epidemiology, and control. Am. J. Med. **108:**290–295.
19. **Kool, J. L., A. E. Fiore, C. M. Kioski, E. W. Brown, R. F. Benson, J. M. Pruckler, C. Glasby, J. C. Butler, G. D. Cage, J. C. Carpenter, R. M. Mandel, B. England, and R. F. Breiman.** 1998. More than 10 years of unrecognized nosocomial transmission of Legionnaires' disease among transplant patients. Infect. Control Hosp. Epidemiol. **19:**898–904.
20. **Lowry, P. W., R. J. Blankenship, W. Gridley, N. J. Troup, and L. S. Tompkins.** 1991. A cluster of *Legionella* sternal-wound infections due to postoperative topical exposure to contaminated tap water. N. Engl. J. Med. **324:**109–113.
21. **Mayock, R., B. Skale, and R. B. Kohler.** 1983. *Legionella pneumophila* pericarditis proved by culture of pericardial fluid. Am. J. Med. **75:**534–536.
22. **McCabe, R. E., J. C. Baldwin, C. A. McGregor, D. C. Miller, and K. L. Vosti.** 1984. Prosthetic valve endocarditis caused by *Legionella pneumophila*. Ann. Intern. Med. **100:**525–527.
23. **Spieker, S., D. Petersen, A. Rolfs, F. Fehrenbach, R. Kuntz, R. H. Seuffer, M. Fetter, and J. Dichgans.** 1998. Acute disseminated encephalomyelitis following Pontiac fever. Eur. Neurol. **40:**169–172.
24. **Stahelin, J., D. Goldenberger, H. E. Gnehm, and M. Altwegg.** 1998. Polymerase chain reaction diagnosis of *Kingella kingae* arthritis in a young child. Clin. Infect. Dis. **27:**1328–1329.
25. **Thacker, W. L., R. F. Benson, J. L. Staneck, S. R. Vincent, W. R. Mayberry, D. J. Brenner, and H. W. Wilkinson.** 1988. *Legionella cincinnatiensis* sp. nov. isolated from a patient with pneumonia. J. Clin. Microbiol. **26:**418–420.
26. **Tompkins, L. S., B. J. Roessler, S. C. Redd, L. E. Markowitz, and M. L. Cohen.** 1988. *Legionella* prosthetic-valve endocarditis. N. Engl. J. Med. **318:**530–535.
27. **Waldor, M. K., B. Wilson, and M. Swartz.** 1993. Cellulitis caused by *Legionella pneumophila*. Clin. Infect. Dis. **16:**51–53.
28. **Warner, C. L., P. B. Fayad, and R. R. Heffner, Jr.** 1991. *Legionella* myositis. Neurology **41:**750–752.