Culture-Negative Endocarditis Due to Houston Complex *Bartonella henselae* Acquired in Noumea, New Caledonia

Dani Rodrick, Belinda Dillon, Mark Dexter, Ian Nicholson, Sebastien Marcel, David Dickeson, and Jon Iredell

Westmead Private Hospital, Centre for Infectious Diseases and Microbiology and Centre for Infectious Diseases and Microbiology Laboratory Services, Institute for Clinical Pathology and Medical Research, Westmead Hospital, Children’s Hospital Medical Centre, Westmead, and University of Sydney, Sydney, New South Wales, Australia, and Service de Neurologie, CHT Gaston Bourret, Nouméa, New Caledonia

Received 13 August 2003/Returned for modification 23 September 2003/Accepted 23 October 2003

A 44-year-old man with a bioprosthetic aortic valve suffered destructive endocarditis with severe embolic disease due to *Bartonella henselae* infection. Multilocus sequence typing was successfully performed with crude preparations of operative tissue as templates, and the infecting organism was determined to be typical of the Houston clonal group, although it was never cultured from blood or tissue. This is the first report of *B. henselae* infection in the South Pacific, and it reminds one that *B. henselae* is a cause of potentially lethal culture-negative endocarditis which may respond poorly to conventional empirical therapy. Nothing is known of the epidemiology of the infection in this region, but it is likely to be common and to contain representatives of both major clonal complexes. This study emphasizes the ease with which multilocus sequence typing can be used directly with tissue, which is important because of suggestions of strain-dependent clinical outcomes.

**CASE REPORT**

A 44-year-old New Caledonia man presented to his local hospital from his home in urban Noumea with the sudden onset of right-sided hemiparesis and aphasia. The patient had undergone bioprosthetic aortic valve replacement for severe aortic regurgitation from a congenital bicuspid valve 2 years earlier without complications or any suggestion of perioperative infection. He was on no immunosuppressive therapy and was not diabetic. His only other medical history was that of essential hypertension, and he recalled no specific history of an inflammatory nodule or marked regional adenopathy or any specific exposure to a cat scratch or bite. His symptoms completely resolved, but he presented again 5 months later with a similar neurological deficit associated with headaches and vomiting. A cerebral computerized tomography scan demonstrated two distinct lesions consistent with embolic infarcts. Transesophageal echocardiography in Noumea failed to detect evidence of endocarditis; however, empirical treatment with vancomycin was commenced after several blood cultures failed to disclose a causative agent.

For neurosurgical assessment, the patient was transferred to Australia, where computerized tomography of the chest and abdomen demonstrated cardiomegaly, a left pleural effusion, and multiple splenic infarcts. Magnetic resonance imaging revealed bilateral cerebral infarcts of various ages associated with localized Wallerian degeneration and a small infarct involving the thoricord. Since an occipital lesion had been complicated by limited hemorrhage, a cerebral angiogram which excluded mycotic aneurysms was performed. The neurosurgical opinion at this time was that these lesions were most consistent with embolic phenomena, implicating the prosthetic valve. All antibiotic therapy was ceased for 48 h on the arrival of the patient in Australia, and three cultures of blood taken during this time were again reported to be negative. Investigations at this time also revealed moderate renal impairment with nephrotic-range (5 g/day) proteinuria, which was attributed to glomerulonephritis. Empirical treatment with fluoxacinil, ciprofloxacin, and rifampin was commenced, but the patient continued to manifest a low-grade fever, progressive anemia (normocytic and normochromic but Coombs positive; total hemoglobin concentration, 7.1 g/dl), and rising inflammatory markers (white blood cell count, 5.3 × 10^9/liter; erythrocyte sedimentation rate, >120 mm/h, C-reactive protein concentration, 71.5 mg/liter). After a week on this treatment, and 3 weeks after the previous negative study, a transthoracic echocardiogram showed moderate mitral regurgitation as well as a large, mobile vegetation on the prosthetic aortic valve.

The patient then underwent a second aortic valve replacement. At surgery, the mitral valve was preserved, but there was extensive involvement of the porcine aortic valve prosthesis and adjacent myocardium with purulent and necrotic tissue, necessitating debridement. The tissue destruction involved the intrinsic conduction system, and the patient ultimately required implantation of a permanent pacemaker. The patient required substantial doses of noradrenaline, vasopressin, and metaraminol to tolerate cardiopulmonary bypass, and his postoperative course was complicated by transient worsening of his renal function, although he did not require renal replacement therapy. The patient remained afebrile postoperatively and was discharged well on azithromycin (500 mg daily) and ciprofloxacin (500 mg twice a day).

**Microbiology and sequence typing.** Three perioperative cultures of blood taken after 48 h without antibiotics in conventional media (BD Bactec Plus Aerobic/F and BD Bactec Plus Anaerobic) were negative. Further blood cultures taken on October 1, 2003, were positive for *B. henselae*.
According to the prototype scheme (18).

lodged for Houston 1 type strain ATCC 49882 (or ST1, sequences obtained for all genes studied were identical to those of Bartonella henselae and serology (9, 33), a cell division protein gene (groEL) (18), and a chaperonin gene (groEL) (18). Sequences were determined by using an ABI 373 Sequencer (Applied Biosystems) and aligned with published sequences from GenBank for the Houston 1 and Marseille strains by using EclustalW (ANGIS). Accession numbers are as follows: 16S, M73229; gltA, L38987; ftsZ, AF061746; ribC, AJ132928; groEL, AF014829. DNA sequences obtained for all genes studied were identical to those lodged for Houston 1 type strain ATCC 49882 (or ST1, according to the prototype scheme [18]).

Clinical Bartonella henselae infection in Australia is well documented (11, 14) and is an important cause of culture-negative bacterial endocarditis. Presented here is a case of serious infection in a man whose only immune compromise appears to be a bioprosthetic (porcine) aortic valve. The illness appears to have progressed over several months without specific treatment, and this case emphasizes the value of serological testing with culture-negative endocarditis. The renal impairment in this case, which was attributed to glomerulonephritis but was not biopsy proven, influenced the choice of ciprofloxacin over an amino-glycoside. In vitro studies of antimicrobial susceptibility suggest that quinolones and tetracyclines may be inadequate (21), but clarithromycin and ciprofloxacin in combination have been used successfully for Bartonella osteomyelitis (35), and azithromycin is highly active in vitro (37). Doxycycline and gentamicin are effective for Bartonella quintana endocarditis (12), although a recent review of Bartonella endocarditis including 12 Bartonella quintana cases among 60 culture-positive cases (mostly B. quintana cases) described a 12% mortality rate overall and suggested poor efficacy of doxycycline alone for Bartonella endocarditis. On the other hand, aminoglycoside therapy for more than 2 weeks in combination with a variety of agents was clearly associated with a better outcome and is recommended as a component of all endocarditis therapies (34).

B. henselae commonly infects cats (5) and humans (11) in developed countries and may be much more common in less developed regions (15). B. henselae prevalence in cats is related largely to flea burdens, and the relationship between the insect vector and the host animal is well described for Bartonella spp. (30). It is therefore predictable that the mild winters and warm, humid summers of New Caledonia, a small Pacific island north of New Zealand and approximately 850 miles east of Australia, would provide a sustainable feline (and flea) population as a zoonotic reservoir. Human B. henselae infection has not previously been reported in the South Pacific, but the ubiquity of B. henselae is emphasized by reports from Hawaii (8), Indonesia (26), Singapore (32), Thailand (28), and the Philippines (7).

Terminal subculture onto chocolate agar in 5% CO2 or into a suitable cell line (e.g., Vero or HEp-2 cells) is generally recommended to isolate B. henselae but may meet with limited success even in expert laboratories (24). Serology results, valuable in both the diagnosis (17) and the management (13) of Bartonella endocarditis, were strongly positive in this case and would have been so earlier in the illness. The organism involved in this case was shown to be a typical Houston-like strain and would be expected to be easily detected serologically by standard methods. However, there are two serotypes (Houston and Marseille) which appear genetically distinct, and serodiagnosis may be problematic. Bartonella and Q fever serology followed by special staining, PCR techniques, and tissue culture may greatly increase diagnostic yield for culture-negative endocarditis (16), but cross-reactivity occurs between Bartonella, chlamydiae, and the Q fever agent (Coxiella burnetii), another important cause of culture-negative endocarditis (25). In addition, serodiagnosis based on the detection of antibodies to Houston 1 antigens in many countries, including Australia (11), may not reliably detect Marseille-like strains (10), which include proven human pathogens in Oceania (9, 19). A recent follow-up study from Israel involving outer membrane preparations of the same strain used in our IFAT (ATCC 49793, a typical Houston-like strain) showed that immunoglobulin M-specific antibody detection in an enzyme immunoassay was especially highly specific in 98 cases of proven cat scratch disease (31), but the serotype specificity of this assay also remains an open question.

16S ribosomal DNA (rDNA) genotypes have traditionally been the basis for genotyping in bacteria and are also useful predictors of serotype (23). Recent Japanese reports have found 16S type I (predictive of Houston-type strains) to be much more common in feline isolates (27), in contrast to other studies which have shown 16S type II (predictive of Marseille type) to dominate among feline isolates (1, 3, 4, 9). Thus, there may be important regional differences, and many sequence combinations represented in European isolates were not described in an Australian strain collection (18). Consistent with other reports, 16S type I rDNA has been shown to be significantly overrepresented in human isolates (9). This finding is especially important because Bartonella strains may vary significantly in their virulence. While some of this variation is clearly due to phase variation and laboratory passage (2, 22), the 16S genotype may itself predict different outcomes for human infection (6).
A variety of DNA fingerprinting techniques have therefore been applied to the epidemiological typing of *B. henselae* (36), but interlabatory comparisons are difficult. Such comparisons are complicated by genomic variation in vitro (22) and in vivo (20), and a variety of gene sequences, including direct typing based on ribosomal sequences in tissue specimens, have been utilized in typing methods in an effort to overcome this difficulty (29). However, noncongruent inheritance of 16S rDNA and highly conserved housekeeping genes such as *gltA* has been reported (9), and more recently, the failure of 16S rDNA to accurately predict clonal grouping (including that of Houston and Marseille complexes) as defined by multilocus sequence typing has been demonstrated (18). This report demonstrates the facility with which accurate genotype assignment can be performed with culture-negative tissues by using multiple genetic loci. While differences in clinical outcomes and relationships between genotype, serotype, and virulence type remain unresolved, it is important to gather clear and unambiguous epidemiological data. Testing of additional genetic loci in large and more geographically representative strain collections should ultimately define an efficient validated multilocus sequence typing method for *B. henselae*. The well-known difficulty in culturing the causative agent (24) need not be a barrier to resolving questions of genotype, serotype, and virulence type relationships if simple methods such as those described herein are used either locally or by transport of tissue specimens to laboratories with an interest in addressing the epidemiology of this pathogen.

REFERENCES