Unique Case of *Helicobacter* sp. Osteomyelitis in an Immunocompetent Child Diagnosed by Broad-Range 16S PCR

Kathryn A. Harris,^{1*} Katy J. Fidler,² John C. Hartley,¹ Julie Vogt,² Nigel J. Klein,² Fergal Monsell,³ and Vas M. Novelli²

Department of Microbiology,¹ Department of Infectious Diseases,² and Department of Orthopaedics,³
Great Ormond Street Hospital for Children NHS Trust, London, United Kingdom

Received 2 November 2001/Returned for modification 2 January 2002/Accepted 27 May 2002

We report the first case of *Helicobacter* sp. osteomyelitis in an immunocompetent child. The infection was diagnosed by broad-range 16S PCR followed by sequencing of the resulting amplicon. All other microbiological investigations proved negative. This case highlights the importance of molecular methods in the diagnosis of unsuspected etiological agents and the potential role of *Helicobacter* sp. in human infection.

CASE REPORT

A 5-year-old girl from Yemen presented to a London hospital with a painful mass in the thigh, fever, and weight loss. The history was that the pain, swelling, and fevers had started simultaneously 8 months earlier, at which time she attended a hospital in Yemen. She initially received 2 weeks of oral antibiotic therapy. In the following 2 months the mass had grown rapidly and two biopsies had been performed; one was said to be consistent with a diagnosis of osteomyelitis and the other with osteosarcoma. Following a further 2 weeks of antibiotics she had been referred to a hospital in Jordan. Two further biopsies were suggestive of osteomyelitis, although microscopy and culture, including cultures for mycobacteria, were negative. She received intravenous antibiotics (imipenem) for 6 weeks, but during the next 5 months she developed daily fevers, anorexia, a weight loss of 5.5 kg, and anemia requiring three blood transfusions. The mass continued to enlarge and she became unable to bear weight.

There was no significant past medical history. Relevant family history included nonconsanguineous, well parents, four well siblings, and a brother who died in infancy of pneumonia.

Examination at our hospital revealed a cachectic, pale child with a fever of 39.4°C. A large 24- by 15-cm hard, tender, warm mass was obvious on the anterolateral aspect of her right thigh. The rest of the clinical examination was unremarkable.

Investigations revealed the following values: hemoglobin, 6.5 g/dl; total white blood cells (WBC), 20,600 cells/mm³ (84% polymorphonuclear leukocytes, 9% lymphocytes, 0.1% eosinophils); platelet count, 1,007,000 cells/mm³; C-reactive protein, 36 mg/dl (0 to 2 mg/dl); erythrocyte sedimentation rate, 135 mm/h (0 to 10 mm/h). Urea and electrolytes and liver function tests were normal. Peripheral blood cultures and a tuberculin test (10 U) were negative. The following immunology investigations were within normal limits: immunoglobulin G (IgG), IgA, IgM, lymphocyte subsets, T-cell activation markers

(CD25, HLA-DR, CD45 isotypes), T-cell proliferation to mitogens, nitroblue tetrazolium test, C3, C4, and total hemolytic complement.

IgE was elevated at 328 kU/liter (0 to 48 kU/liter). Radiographs of the right femur revealed extensive spiculated new bone formation (Fig. 1A). Magnetic resonance imaging of the leg showed marked periosteal reaction of the femur and a large circumferential soft tissue mass extending from the upper right femoral neck to the suprapatellar region (Fig. 1B). Isotope bone scan showed very intense activity of the right femur and multifocal bony abnormalities consistent with infection. Bone marrow aspirate and trephine biopsy showed no evidence of malignancy. Biopsy of the lesion revealed an acute and chronic inflammatory infiltrate consistent with active osteomyelitis. There were no granulomas and no evidence of malignancy. Initial microbiological examinations of biopsy material revealed no organisms on a Gram stain and no acid-fast bacilli by Ziehl-Neelsen staining. Routine bacterial and mycobacterial cultures were negative. DNA was extracted from 20 mg of the lesion using the QIAmp tissue kit (Qiagen, Crawley, West Sussex, United Kingdom) according to the manufacturer's instructions. A 320-bp fragment of the bacterial 16S gene was amplified from 5 µl of this extract by using the primers 16SFa (5'-GCTCAGATTGAACGCTGG), 16SFb (5'-GCTCAGGAYGAACGCTGG), and 16SR (5'-TACTGCTGCCTCCCGTA), which are based on sequences that are highly conserved across the bacterial kingdom (C. Linton, personal communication). Cycling parameters were as follows: 94°C for 3 min followed by 26 cycles of 94°C for 30 s, 63°C for 1 min, and 72°C for 1 min. A final extension was carried out at 72°C for 5 min. Amplicons were detected by gel electrophoresis and ethidium bromide staining. The PCR was previously shown to detect 100 CFU per PCR when either *Staphylococcus aureus* or *Escherichia coli* Qiagen-extracted cultures were used as template (K. Harris, unpublished data). An amplicon of the expected size was obtained from both the lesion and the positive PCR control but not from the negative extraction or PCR controls. The DNA sequence of the amplicon from the lesion was determined and compared to the GenBank database by using the BLAST program available at the website of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>). The

* Corresponding author. Mailing address: Department of Microbiology, Level 4 Camelia Botnar Laboratories, Great Ormond Street Hospital for Children NHS Trust, Great Ormond Street, London, WC1N 3JH, United Kingdom. Phone: 44 207 405 9200. Fax: 44 207 813 8268. E-mail: harrik2@gosh.nhs.uk.

FIG. 1. (A) Radiograph of the right femur of a 5-year-old girl with chronic *Helicobacter* sp. osteomyelitis showing extensive spiculated new bone formation along the whole shaft with associated soft tissue swelling. (B) Magnetic resonance image of the right leg of a 5-year-old girl with chronic *Helicobacter* sp. osteomyelitis showing marked periosteal reaction of the femur and a large circumferential soft tissue mass extending from the upper right femoral neck to the suprapatellar region.

closest match was with an isolate named "*Helicobacter rappini*" (GenBank accession no. AF286052) (4, 9).

In light of this result, further cultures were performed from biopsy material (after storage for 7 days at 4°C) on blood and chocolate agars at 37°C in a microaerophilic environment. There was no growth after 10 days.

The patient was treated with intravenous ciprofloxacin, 5 mg/kg twice a day, and clindamycin, 7.5 mg/kg three times a day, for a total of 4 weeks, to continue for 1 year orally. Rapid improvement was seen with a reduction of the erythema and tenderness of the thigh and a decrease of inflammatory markers. With nutritional and physiotherapy support she gained 4 kg in 1 month and started mobilizing. Follow-up at 6 months revealed marked clinical and radiological improvement. Laboratory investigations showed a normal total WBC of 4,700 cells/mm³ and an erythrocyte sedimentation rate of 25 mm/h (0 to 10). At 14 months, her physicians were contacted in Yemen, and they reported that she had improved remarkably, with no fever or pain and a great reduction in the size of the mass.

Further molecular investigations were performed on the DNA extract. The entire 16S gene, approximately 1,400 bp,

was amplified by PCR using primers S-D-Bact-008-a-20 and S-*-Univ-1492-b-A-21 (8) and sequenced in both directions using these and six other sequencing primers. A BLAST search was performed with this full-length sequence and, again, the closest match was found with the same "*H. rappini*" isolate (4). The 16S sequences of the most closely related sequences (determined by BLAST) and representative species within the *Helicobacter* genus were retrieved from GenBank and aligned with our longer sequence (*Helicobacter* sp. GOSH) using the CLUSTAL W algorithm. Only 1,326 bp, the length of the shortest sequence, were analyzed. A dendrogram showing how *Helicobacter* sp. GOSH is related to other *Helicobacter* sequences (Fig. 2) and the percentage divergence between sequences (data not shown) were produced using the MegAlign program (DNASTAR, Madison, Wis.). *Helicobacter* sp. GOSH clustered tightly with several sequences (Fig. 2), including seven unnamed *Helicobacter* spp., six *Helicobacter cinaedi* sequences (four of which are not shown in Fig. 2), a "*Helicobacter westmeadii*" sequence, and an "*H. rappini*" sequence. The percentage divergences between the sequences in this cluster ranged between 0.2 and 0.9%. *Helicobacter* sp. GOSH is most closely related (98.8%) to "*H. rappini*" (GenBank accession no. AF286052) (9), *H. cinaedi* (GenBank accession no. AF396080),

FIG. 2. Unrooted tree to show how *Helicobacter* sp. GOSH is related to other members of the genus. The dendrogram was produced using the CLUSTAL W algorithm in the MegAlign program.

and two unnamed *Helicobacter* spp. (GenBank accession nos. AJ249852 and AJ249853).

Discussion. Osteomyelitis in children is principally caused by *S. aureus*, *Streptococcus pneumoniae*, beta-hemolytic streptococci, *Haemophilus influenzae*, *Enterobacteriaceae*, and mycobacteria. Identification of the causative organism from blood cultures or bone biopsy, and appropriate antibiotic selection, is fundamental to successful management. Inadequate or delayed therapy may result in chronic osteomyelitis with severe consequences. We have described a child with an extensive chronic lesion of the leg and a severe debilitating illness in whom the diagnosis could not be determined after repeated investigation with conventional microbiological and histological methods. The use of broad-range 16S PCR and sequencing revealed *Helicobacter* as the putative etiological agent, confirming the diagnosis of chronic osteomyelitis and enabling appropriate antibiotic therapy to be instituted.

Identification of species within the *Helicobacter* genus is often difficult (2, 3, 7), with potential misidentification of *H. cinaedi* isolates when 16S gene sequences alone are used as a method of classification (12). *Helicobacter* sp. GOSH is closely related to *H. cinaedi* and "*Helicobacter flexispira*" (also known as "*H. rappini*"), based on 16S ribosomal DNA sequences (Fig. 2). However, we have not attempted to assign a species name to our sequence.

The majority of reported infections with *H. cinaedi*-like organisms occur in immunocompromised patients. "*H. westmeadii*," "*H. flexispira*," and several unnamed *Helicobacter* species have been isolated from the blood of patients with immunodeficiencies (9, 11, 13, 14). There are two reported cases of skin and bone infections with "*H. flexispira*"; both of these were in patients with X-linked agammaglobulinemia (1). Our immunological investigations failed to demonstrate a defect in either

humoral or cellular immunity and are compatible with her healthy premorbid condition. There are a few reports of *H. cinaedi*-like organisms causing infection in immunocompetent patients; these include *H. cinaedi* in septic arthritis and bacteremia (6) and "*H. flexispira*" in a bacteremia (10). This demonstrates the potential pathogenic role of *Helicobacter* spp. in both immunocompromised and immunocompetent individuals.

The significance of bacterial DNA in a bone lesion when culture results are negative is still unknown. However, we feel that in this case a *Helicobacter* sp. is the etiological agent of the osteomyelitis and there are several pieces of evidence to support this. The PCR on the lesion was more strongly positive, as determined by relative band intensities, than the controls that contained genomic DNA from approximately 100 CFU/PCR of *S. aureus* and *E. coli*. Only DNA from a single bacterial species was detected. *Helicobacter* spp. are not routinely cultured in our laboratory and are unlikely to be contaminants. In addition to this, the clinical history, histological appearance, and inflammatory markers all suggested an infectious cause. Failure of earlier biopsies to yield an organism and failure to respond to earlier antibiotic treatment suggest that common bacterial agents are not involved. Finally, a good response to an extended course of alternative antimicrobials which may be expected to be active in some *Helicobacter* spp. infections (9, 10, 14) indicates that the diagnosis of *Helicobacter* sp. osteomyelitis was correct. Failure to culture the organism can be explained by an inadequate initial procedure followed by an extended delay in establishing a microaerophilic environment.

We have described the use of a broad-range 16S PCR to diagnose a *Helicobacter* sp. osteomyelitis when all other investigations proved negative. One other report has described the use of 16S PCR and sequencing as the sole method of detec-

tion of a *Helicobacter* sp. directly from a clinical specimen (5). This highlights the potential of such molecular methods in the diagnosis of infection with difficult-to-culture organisms, especially when the pathogen is rare or totally unsuspected as the etiological agent. The true number of infections with *Helicobacter* spp. in humans could be much greater than previously reported and indicates that further studies are required to evaluate the true extent of *Helicobacter* spp. in pathogenic childhood infections.

Nucleotide sequence accession number. The *Helicobacter* sp. GOSH 16S ribosomal DNA sequence was deposited in GenBank under accession number AF362544.

This work was undertaken by the Great Ormond Street Hospital for Children NHS Trust, who received a proportion of its funding from the NHS Executive.

REFERENCES

1. Cuccherini, B., K. Chua, V. Gill, S. Weir, B. Wray, D. Stewart, D. Nelson, I. Fuss, and W. Strober. 2000. Bacteremia and skin/bone infections in two patients with X-linked agammaglobulinemia caused by an unusual organism related to *Flexispira/Helicobacter* species. *Clin. Immunol.* **97**:121–129.
2. Dewhirst, F. E., J. G. Fox, E. N. Mendes, B. J. Paster, C. E. Gates, C. A. Kirkbride, and K. A. Eaton. 2000. "*Flexispira rappini*" strains represent at least ten *Helicobacter* taxa. *Int. J. Syst. Evol. Microbiol.* **50**:1781–1787.
3. Dewhirst, F. E., J. G. Fox, and S. L. On. 2000. Recommended minimal standards for describing new species of the genus *Helicobacter*. *Int. J. Syst. Evol. Microbiol.* **50**:2231–2237.
4. Drancourt, M., C. Bollet, A. Carlioz, R. Martelin, J. P. Gayral, and D. Raoult. 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J. Clin. Microbiol.* **38**:3623–3630.
5. Han, S. R., C. Schindel, R. Genitsariotis, E. Marker-Hermann, S. Bhakdi, and M. J. Maeurer. 2000. Identification of a unique *Helicobacter* species by 16S rRNA gene analysis in an abdominal abscess from a patient with X-linked hypogammaglobulinemia. *J. Clin. Microbiol.* **38**:2740–2742.
6. Lasry, S., J. Simon, A. Marais, J. Pouchot, P. Vinceneux, and Y. Boussou-gant. 2000. *Helicobacter cinaedi* septic arthritis and bacteremia in an immunocompetent patient. *Clin. Infect. Dis.* **31**:201–202.
7. Solnick, J. V., and D. B. Schauer. 2001. Emergence of diverse *Helicobacter* species in the pathogenesis of gastric and enterohepatic diseases. *Clin. Microbiol. Rev.* **14**:59–97.
8. Suau, A., R. Bonnet, M. Sutren, J. J. Godon, G. R. Gibson, M. D. Collins, and J. Dore. 1999. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl. Environ. Microbiol.* **65**:4799–4807.
9. Tee, W., A. Jenney, A. McPhee, A. Mijch, and M. Dyall-Smith. 2001. "*Helicobacter rappini*" isolates from two homosexual men. *Clin. Infect. Dis.* **33**:E8–E11.
10. Tee, W., K. Leder, E. Karroum, and M. Dyall-Smith. 1998. "*Flexispira rappini*" bacteremia in a child with pneumonia. *J. Clin. Microbiol.* **36**:1679–1682.
11. Trivett-Moore, N. L., W. D. Rawlinson, M. Yuen, and G. L. Gilbert. 1997. *Helicobacter westmeadii* sp. nov., a new species isolated from blood cultures of two AIDS patients. *J. Clin. Microbiol.* **35**:1144–1150.
12. Vandamme, P., C. S. Harrington, K. Jalava, and S. L. W. On. 2000. Misidentifying helicobacters: the *Helicobacter cinaedi* example. *J. Clin. Microbiol.* **38**:2261–2266.
13. Weir, S. C., B. Cuccherini, A. M. Whitney, M. L. Ray, J. P. MacGregor, A. Steigerwalt, M. I. Daneshvar, R. Weyant, B. Wray, J. Steele, W. Strober, and V. Gill. 1999. Recurrent bacteremia caused by a "*Flexispira*"-like organism in a patient with X-linked (Bruton's) agammaglobulinemia. *J. Clin. Microbiol.* **37**:2439–2445.
14. Weir, S. C., C. L. Gilbert, F. M. Gordin, S. H. Fischer, and V. J. Gill. 1999. An uncommon *Helicobacter* isolate from blood: evidence of a group of *Helicobacter* spp. pathogenic in AIDS patients. *J. Clin. Microbiol.* **37**:2729–2733.