Refractory Chronic Pleurisy Caused by *Helicobacter equorum*-Like Bacterium in a Patient with X-Linked Agammaglobulinemia[▽]

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Received 9 March 2011/Returned for modification 6 April 2011/Accepted 6 June 2011

We describe a 35-year-old man with X-linked agammaglobulinemia who had refractory chronic pleurisy caused by a *Helicobacter equorum*-like bacterium. Broad-range bacterial PCR targeting the 16S and 23S rRNA genes and *in situ* hybridization targeting the 16S rRNA gene of *H. equorum* confirmed the presence of this pathogen in a human for the first time.

CASE REPORT

A 35-year-old man was referred to us for a low-grade fever, fatigue, and discomfort in the right thorax. He had been diagnosed with X-linked agammaglobulinemia (XLA) during the first year of life (5). Upon diagnosis, he showed extremely low serum immunoglobulin G, A, and M levels (650 mg/liter, under 80 mg/liter, and under 60 mg/liter, respectively) and had a missense mutation, L111R (464T>G), in Bruton's tyrosine kinase (BTK) gene (Fig. 1A). His family pedigree with respect to XLA is shown in Fig. 1B. Substitution therapy with intravenous immunoglobulin was administered every 4 weeks from his childhood. During his high school years, he had acute right pleurisy and a pleural puncture, but the details are unclear. In 2006, he suffered from right pleurisy again and then was repeatedly admitted to our hospital for 2 years. No pathogens causing his chronic pleurisy have ever been detected, even though some conventional cultures of blood and sputum have been performed. However, administration of panipenem/betamipron (PAPM/BP) had been the only way to improve his chest discomfort and transiently reduce C-reactive protein (CRP) levels. Administration of other antimicrobial treatments, such as the use of macrolides, cepheems, newquinolones, glycopeptides, and carbapenems other than PAPM/BP, has resulted in no improvement.

On admission in 2008, his laboratory findings showed a normal white blood cell count (7.76×10^9 /liter), a high CRP level (50.3 mg/liter), and a very high endotoxin level (131 ng/liter). A chest radiograph showed a thickened right pleura and pneumonia in the right inferior lung (Fig. 2A), and a computed tomography (CT) scan of the chest also showed the thickened right pleura with calcification and an alveolar opacity in the

right inferior lung (Fig. 2B). We suspected a Gram-negative bacterial infection due to the transient PAPM/BP effectiveness and the high endotoxin level or *Mycobacterium tuberculosis* complex (MTC) infection due to the CT calcification finding. However, conventional cultures of blood, urine, sputum, and feces were all negative, as they had often been in the past.

A transbronchial lung biopsy and a transcutaneous pleural biopsy were performed for definite diagnosis. Histological examination of the alveolar spaces in the right lung showed intraluminal fibrosis of distal airspaces with foamy alveolar macrophages, suggesting secondary organizing pneumonia (OP) (Fig. 2C). In addition, examination of the right pleura showed chronic inflammation (Fig. 2D). Despite these findings, conventional bacterial cultures of biopsy samples from the right pleura grown in sheep blood agar (Nissui Pharmaceutical, Tokyo, Japan) and chocolate agar (Eiken Kagaku, Tokyo, Japan) plates showed no evidence of infection, and MTC cultures grown in an egg-based solid medium (Ogawa medium) (Kyokuto Pharmaceutical, Tokyo, Japan) was also negative.

To determine the pathogen of this refractory pleurisy, we performed broad-range bacterial PCR and mycobacterial PCR using the pleural samples. The PCR products targeting the bacterial 16S and 23S rRNA genes revealed a 1,473-bp band and a 563-bp band, respectively (Fig. 3A). Sequencing analysis was carried out using a GenBank BLAST search (National Center for Biotechnology, Bethesda, MD). Sequence editing and phylogenetic analyses were performed with ClustalW. The sequence of a 1,473-bp fragment of 16S rRNA gene confirmed the presence of *Helicobacter equorum*-like (99.8% identical) bacterium DNA (GenBank accession no. AB571486). Moreover, the sequence of a 563-bp fragment targeting the bacterial 23S rRNA gene was 98.9% similar to that of *H. equorum* (GenBank accession no. AB571487). On the other hand, PCR amplification of the 16S rRNA gene for MTC determinations was negative.

Next, to further demonstrate that the pleural infection involved an *H. equorum*-like bacterium, we performed *in situ* hybridization

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[▽] Published ahead of print on 15 June 2011.

FIG. 1. Molecular analysis of the BTK gene and pedigree of our patient. (A) A missense mutation, L111R in BTK gene, identified in our patient. (B) The family tree for our patient. An arrow indicates the proband with XLA. The solid squares denote patients with XLA; circles with black dots denote mutation carriers.

using a digoxigenin-labeled single-strand RNA probe. The probe for positions 999 to 1118 in the 16S rRNA gene of *H. equorum* (GenBank accession no. AM998804) was designed as follows: 5'-UCCUCACCUUCCUCCUCCUACGAAGGCAGUCUC CUUAGAGUGCUCAGCCAAACUGCUAGCAACUAAGG ACGAGGGUUGCGCUCGUUGCGGGACUUAACCCAAC AUCUCACGACACGAGC-3'. Positive signals in the pleural sample were confirmed with a nitroblue tetrazolium/5-bromo-4-chloro-3-indolylphosphate (NBT/BCIP) system (Roche Diagnostics, Tokyo, Japan) (Fig. 3B) but not in the control, which included the sense probe (Fig. 3C). These results indicated that the refractory chronic pleurisy in our patient was caused by an *H. equorum*-like bacterium, which in turn caused the development of secondary OP.

We began administration of PAPM/BP at a high dose of 8 g/day and of clarithromycin orally for 2 months. Since then, the patient has had no symptoms, and tests have shown negative CRP results and an endotoxin level of less than 10 ng/liter.

Since the discovery of *Helicobacter pylori* in 1984 (7), various *Helicobacter* species have been described in a wide variety of animal hosts, and transmission to humans has been suggested (3, 10, 14). In general, *Helicobacter pylori* is associated with gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma (3). Also, non-*H. pylori* *Helicobacter* species are associated with gastric, intesti-

FIG. 2. Imaging and histological findings of refractory chronic pleurisy and secondary OP. (A and B) Results of a chest radiograph (A) and a chest CT scan (B) upon the latest admission of the patient to the hospital. (C) Histological finding in alveolar spaces, showing intraluminal fibrosis (arrow) (hematoxylin and eosin). (D) Histological finding in the right pleura, showing chronic inflammation (hematoxylin and eosin).

FIG. 3. Detection of *H. equorum*-like bacterium DNA in samples from the right pleura. (A) Broad-range bacterial products from a PCR targeting the 16S rRNA gene (left) and the 23S rRNA gene (right) determined using biopsy samples from the right pleura. S1 and S2 denote DNA samples from our patient. One-tenth the amount used in S1 was used in S2. N, negative control; M, marker. (B) Result of *in situ* hybridization of the pleural samples performed using the probe for the 16S rRNA gene of *H. equorum*. Scale bar, 500 μ m. (B') Higher magnification of the bracketed areas shown in panel B. The signals of *H. equorum* were detected (arrowheads). Scale bar, 100 μ m. (C) Negative control. Scale bar, 500 μ m.

nal, and hepatobiliary diseases in humans (3, 10, 14). This understanding is attributed to molecular diagnosis based on the sequencing of bacterial 16S and 23S rRNA genes, an analytical technique that has already proved useful for various bacterial infections during antimicrobial treatment (11), for rare or unexpected pathogens (11), and particularly for difficult-to-culture bacteria such as non-*H. pylori* *Helicobacter* species (3). Herein, we have also described a case of refractory chronic pleurisy caused by an *H. equorum*-like bacterium that was subjected to molecular analysis.

Our patient had XLA, which is a rare genetic disorder of B-cell maturation characterized by the absence of mature B cells, very low serum levels of all immunoglobulin isotypes, and a lack of specific antibody production (6). He suffered for 2 years from right chronic pleurisy due to an unknown pathogen. We treated him with PAPM/BP on the basis of the clinical findings, but we were confused because the efficacy was transitory. Molecular diagnosis targeting bacterial 16S and 23S rRNA genes revealed that only DNA of an *H. equorum*-like bacterium that has not previously been reported to have been found in samples from humans was isolated from biopsy samples of our patient. Unfortunately, a culture for the *Helicobacter* species could not be performed for our patient because of the unexpected bacterium, but such culture is also difficult to perform in general, particularly for non-*H. pylori* *Helicobacter* species (3). We therefore performed *in situ* hybridization using the probe for the 16S rRNA gene of *H. equorum* and thereby confirmed that the infection had been caused by an *H. equorum*-like bacterium.

H. equorum, which is a Gram-negative, curved, and motile bacterium, was recently isolated from horse feces by molecular diagnosis (8). Additional investigation revealed that the prevalence of *H. equorum* was significantly higher in horses under veterinary care than in healthy horses, and *H. equorum* DNA

has never been detected in human samples (9). To the best of our knowledge, this is the first case of infection with *H. equorum*-like bacterium in a human with XLA and in the respiratory system. So far, *Helicobacter* infections in patients with XLA have rarely been reported (2, 4, 12, 13), and none of those reported have been due to the presence of *Helicobacter* species in the respiratory system. Freeman and Holland illustrated the importance of humoral immunity in *Helicobacter* infections involving mucosal surfaces, because patients with XLA have been prone to chronic bacteremia, skin infections, and bone infections by the *Helicobacter* species (1). Our patient with XLA showed no evidence of bacteremia or other infections due to the presence of an *H. equorum*-like bacterium. In addition, the studied patient had not had any contact with horse feces, which is a possible vector of *H. equorum*, for the previous 2 years, though he had a history of right pleurisy. Finally, the source of the infection in our patient could not be identified, but we think it would be accurate to say that this infection, which exhibited abnormal humoral immunity, may have been associated with XLA.

Our patient with XLA has been treated with PAPM/BP, but we are unsure as to which antimicrobial treatment to use in a case like this. Because of the difficulty of performing culture, *in vitro* susceptibility testing has scarcely been evaluated or standardized for *H. equorum*. Moyaert et al. reported resistance to cephalotin and nalidixic acid and sensitivity to metronidazole for *H. equorum* (8). We also noted evidence of multiple drug resistance of this organism clinically, as our patient improved only after treatment with PAPM/BP; administration of many other antimicrobial treatments resulted in no improvement. Further investigation is needed, because antimicrobial treatment for *H. equorum* may be difficult.

In conclusion, we have described a case of chronic pleurisy associated with the presence of an *H. equorum*-like bacterium.

All of the clinical findings for our patient—transient PAM/BP effectiveness, a high serum endotoxin level, and imaging-histological findings of chronic inflammation—were consistent with infections by this organism. This case illustrates both the usefulness of molecular diagnosis of infections with unknown organisms and the pathogenicity of the *H. equorum*-like bacterium in immunocompromised humans. In the future, the issues of whether *H. equorum* is associated with diseases in immunocompetent humans or not and of how patients infected with *H. equorum* are to be treated need to be investigated.

ACKNOWLEDGMENTS

We thank Yoshinobu Hirose, Yasushi Ohno, Kouyou Shirahashi, and Hisahi Iwata for contributing to the diagnosis and Hideki Hiraiwa for technical support with the *in situ* hybridization.

This work was supported by a grant from the Ministry of Health, Labor, and Welfare of Japan.

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