Cat Scratch Disease
A Case Confirmed by Nested Polymerase Chain Reaction

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Cat scratch disease (CSD) is the most common cause of localized chronic lymphadenopathy in children and adolescents in the US, but it is rarely reported in Taiwan. It is transmitted by Bartonella henselae. The diagnosis of CSD is usually made by clinical symptoms and compatible pathological findings. Recently, a serum antibody for B. henselae has been used to confirm the diagnosis; it is present in 88 to 100% patients. We report a 19-year-old female who bred cats and developed a painful inguinal node unresponsive to oral medicine. Biopsy showed a reactive lymph node with many epithelioid cell granulomas with central stellate suppurative necrosis and multinucleated giant cells. However, serum antibody to B. henselae by indirect immunofluorescence antibody test (IFA) was negative four weeks after onset of her symptoms. B. henselae DNA was finally detected in the lymph node biopsy specimen by means of nested polymerase chain reaction (PCR) and DNA sequence analysis, confirming the diagnosis of CSD. (Dermatol Sinica 22 : 35-40, 2004)

Key words: Cat scratch disease, Bartonella henselae, Indirect immunofluorescence antibody test (IFA), Nested polymerase chain reaction (PCR)

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INTRODUCTION

Cat scratch disease (CSD), the most common form of localized chronic lymphadenopathy in children and adolescents in the US, is rarely reported in Taiwan. The true incidence of CSD in Taiwan is unknown. It may be more common than is thought if it is underdiagnosed because patients or their doctors ignore it. The overwhelming majority of CSD is caused by Bartonella henselae. Significant titers of B. henselae antibodies are found in 88 to 100% patients who meet the clinical definition of CSD. There are other confirmatory tests for CSD, including a skin test, serology, B. henselae culture, and PCR assay. Serological testing for the presence of antibodies to B. henselae is a widely accepted diagnostic tool for laboratory confirmation of the diagnosis. We report a case of CSD whose diagnosis could not be confirmed by serum antibody but proved later by nested PCR and DNA sequencing.

CASE REPORT

A 19-year-old female suffered from a painful left inguinal mass for 4 days before presenting to our outpatient department. On examination, there was a single flesh-colored tender mass about 2 x 2 cm in size in her left inguinal area. There was no fever or other constitutional symptoms. The initial impression was a subcutaneous tumor with secondary infection, and cephalexin 500 mg (Cephalexin®, SinTong, Taiwan) and the non-steroidal anti-inflammatory drug diclofenac 25 mg (Cataflam®, Novartis, Switzerland) were given. The lesion persisted one week later in spite of the medication. Therefore, an excisional biopsy was performed. Pathologic examination showed an enlarged lymph node with multiple epithelioid granulomas, central suppurative necrosis, and numerous neutrophils. Several suppurative foci had coalesced to form an abscess with a stellate outline. Multinucleated giant cells were also found in the central necrotic area of a granuloma. Acid-fast, PAS, and Warthin-Starry stains did not reveal any definite

Fig. 1
Pathologic examination of an enlarged lymph node showing multiple epithelioid granulomas with central suppurative necrosis and numerous neutrophils. Several suppurative foci have coalesced to form an abscess with a stellate outline (A). Close view of the central suppurative necrosis area showing numerous neutrophils and surrounding epithelioid cells (B).

Fig. 2
Multinucleated giant cells are also found in the central necrosis of a granulomatous inflammatory area.
At that point, a more careful history was taken, and it was found that she had bred cats for 2 years. She did not remember a specific scratch on her left lower extremity, but a reddish papule was found on the left thigh that was thought to be a cat scratch (Fig. 3), leading to consideration of CSD. An indirect immunofluorescence test for IgG antibody (IFA) to *B. henselae* was negative when checked four weeks after the onset of the lesion. Polymerase chain reaction (PCR) and *B. henselae* DNA sequencing were then performed on the biopsy specimen.

To extract DNA from the tissue block, paraffin sections were transferred directly into PCR tubes and incubated, centrifuged, resuspended, spun down, and lyophilized. The pellets were then processed using a Puregene DNA isolation kit (Genta, Minneapolis, MN) according to the manufacturer's instructions. Two pairs of oligonucleotide primers were used to amplify *B. henselae* partial ribD, ribC and ribE genes by nested PCR. The outer primers were PBH-L1 (GATATCGGTGTTGTTGAAGA) and PBH-L2 (AATAAAAGGTATAAAACGCT) and the inner primers were Hensela-A (ATTCAATCCTTTAAAGCAGGGGA) and Hensela-B (TTTGTAGGAAAAACGACTGCA). PCR was carried out in a DNA thermal cycler (GeneAmp PCR System 9700; Perkin-Elmer; Foster City, CA). The first reaction protocol with PBH-L1 and PBH-L2, performed in a microcentrifuge tube, was as follows: (1) denaturation at 95°C for 10 min; (2) amplification for 45 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min; and (3) extension at 72°C for 10 min. Nested PCR was initiated by using 1 μL amplified product from the outer primer set and performed using the same thermal cycler protocol as above for 40 cycles. A positive result was indicated by the appearance of a single band of 345 bp in the specimen (Fig. 4).

The PCR products were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and ABI Prism 377 Genetic Analyzer (PE Applied Biosystems, Foster City, CA). Our sample had a 100% match with the *B. henselae* sequence.

The diagnosis of CSD was thus confirmed. The patient was then followed without any medication for six months. The suspected primary lesion subsided a few days after lymph node excision. The patient refused a second blood test.
for serology but remained free of disease six months after presentation.

DISCUSSION

Cat scratch disease (CSD) was first described by Robert Debre in 1931. It consists of a primary lesion, followed by local lymphadenitis and is often accompanied by systemic symptoms such as fever and fatigue. About 10% of CSD patients have atypical manifestations, including Parinaud's oculoglandular syndrome, erythema nodosum, neuroretinitis, arthritis, pneumonia, granulomatous hepatitis and encephalitis. Usually, the primary lesion regresses spontaneously in less than 2 weeks, leaving a small scar in up to 90% of patients. A single enlarged lymph node is found in 85% of patients, although multiple nodes are occasionally involved. Initially, *Afipia felis* was thought to be the cause of CSD. During the 1990s, however, it was demonstrated conclusively that *B. henselae* (at that time called *Rochilimaea henselae*) is the cause. More recently, two new *Bartonella* species, *B. clarridgeiae* and *B. koehlera*, have been identified in the cat reservoir. The role of these species in the etiology of CSD still needs to be confirmed. It was believed that cats play an important role in the transmission of the disease. Cats may transmit bacteria by bites, scratches or possibly through fleas (*Ctenocephalides felis*).

In patients with regional lymphadenitis, the differential diagnosis includes both infectious and noninfectious causes. Infectious diseases that mimic CSD include infectious mononucleosis, toxoplasmosis, cytomegalovirus infection, lymphogranuloma venereum, and various fungal infections. Noninfectious entities include congenital and acquired cysts, sarcoidosis, Kawasaki disease, and neoplasms. A careful history, differentiation of symptomatology, and appropriate serology for toxoplasmosis, EBV, and CMV will narrow the differential diagnosis.

To diagnose CSD, at least three of the following four criteria have traditionally been required: (1) contact with a cat and the presence of a scratch or a primary lesion; (2) negative studies for other causes of lymphadenopathy; (3) a positive skin test; and (4) characteristic histopathological changes. However, a history of cat contact is not always present, and the skin test is not standardized and widely used. Histopathological findings often reveal a non-specific suppurative granulomatous infiltration. Although *Bartonella* can be identified by Warthin-Starry silver stain, the number of bacilli may be low, leading to difficulty in precise interpretation. Isolation of *B. henselae* in culture is difficult and time-consuming, usually requiring more than two weeks for isolation. Accurate and early diagnosis of CSD is important because it is usually a benign and self-limited disease, whereas prolonged lymphadenopathy may resemble lymphoma or other malignancy. To rule out the possibility of malignancy, patients with regional lymphadenitis often undergo extensive, costly and invasive examinations. Thus, a rapid, easily performed test for CSD is important in order to avoid unnecessary testing.

In 1992, Regnery et al. described an IFA test for detection of anti-*B. henselae* IgG. Since then, IFA has become the most commonly used diagnostic tool for CSD. Several other serological tests, such as enzyme-linked immunosorbent assay (ELISA), and Western blot (WB) analysis, have also been developed, but they have varying sensitivities and specificities. The cut-off value in most IFA assays is 1:64, and reported sensitivity varies from 88% to nearly 100%, with a specificity of 97%. Therefore, many authors regard the serological test is the first step towards confirmation of suspected CSD. Highest antibody titers are usually found 0 to 16 weeks after the onset of lymphadenopathy and decline to borderline levels after 25 to 28 weeks. A four-fold titer decrease from 1:1024 to 1:256 has been noted within the first four weeks in some reports. Therefore, it appears that the highest humoral response detected by IFA occurs early. On the other hand, seronegative results have been found occasionally very early in the course of disease.
and false-negative results may be due to a delayed blood test or infection with other Bartonella species and strains. The first case of CSD in Taiwan was reported by Lee, et al. in 1998 and was diagnosed by traditional criteria and positive serology. In our patient, the negative serologic findings at 4 weeks after onset is uncommon.

PCR assay is an alternative test that directly detects B. henselae DNA in blood, pus or biopsy samples from patients with suspected CSD. Broad-range PCR, using the bacterial 16S rRNA gene sequences as a primer, was first used to detect Bartonella in a patient with bacillary angiomatosis. Broad-range PCR with sequencing was thus developed as an effective way to identify Bartonella spp. In our case, use of outer and inner primers allowed amplification of a specific B. henselae gene sequence, leading to the correct diagnosis. Generally speaking, PCR with appropriate laboratory control has the advantage of providing rapid diagnosis without depending on detection of a patient's humoral immune response.

Because most CSD follows a benign and self-limited course, therapy, if required, generally is supportive. Antibiotic therapy should only be considered in severe illness or in the presence of immunodeficiency. In complicated CSD, treatment with trimethoprim-sulfamethoxazole, ciprofloxacin, or azithromycin is recommended, with gentamicin being reversed for the severely ill patient. In our patient, the suspected primary lesion subsided a few days after lymph node excision. The patient without further anti-infective therapy remained free of disease six months after presentation.

In conclusion, the diagnosis of CSD should be suspected in the presence of a typical clinical presentation. In patients with a negative serologic response, PCR may be used as an alternative diagnostic tool, thus avoiding unnecessary invasive testing or therapeutic intervention. A definite diagnosis can reduce patient anxiety since they can be reassured that they have a benign, self-limited disease.

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REFERENCES

