Isolation in Endothelial Cell Cultures of Chlamydia trachomatis LGV (Serovar L2) from a Lymph Node of a Patient with Suspected Cat Scratch Disease

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An inguinal lymph node, removed from a 21-year-old Romanian man suspected of having cat scratch disease, was sent to our laboratory for Bartonella culture. Lymph node specimens were inoculated on blood-enriched agar and in an endothelial cell culture system using the centrifugation shell vial technique. Bacteria were grown in cell monolayers and detected as positive with an anti-Bartonella henselae rabbit serum. However, such bacteria were identified as Chlamydia trachomatis biobar LGV serovar L2 by PCR sequencing techniques.

As a reference laboratory for rickettsial diseases, we have developed a centrifugation cell culture system in shell vials which we use routinely for isolation of rickettsial pathogens in a biosafety level 3-equipped laboratory. Species of the genus Rickettsia are grown in Vero cells and are usually recovered from blood and skin biopsy specimens (16, 20). Coxiella burnetii is cultured in human embryonic lung fibroblasts and may be grown from blood, liver biopsy specimens, and cardiac valve specimens removed from patients with Q fever endocarditis (19). Bartonella species are grown in endothelial cells (ECV 304) and have mainly been recovered from blood, lymph node specimens (especially in patients with cat scratch disease), biopsy specimens from cutaneous bacillary angiomatosis lesions, and cardiac valve specimens from patients with endocarditis (15, 21). However, using the same endothelial cell system in shell vials, we recently isolated other pathogens, such as Francisella tularensis (6), Legionella pneumophila (14), and Mycobacterium sp. (unpublished data) from lymph node specimens, which were originally cultured in an attempt to recover Bartonella henselae, the agent of cat scratch disease. We presently report the isolation of Chlamydia trachomatis biobar LGV, using the same shell vial system, from inguinal lymph node specimens removed from a patient with typical stage 2 lymphogranuloma venereum (LGV).

MATERIALS AND METHODS

Case patient. A 21-year-old immunocompetent man from Romania was admitted to a hospital in Marseille, in the south of France, on 3 July 1998, while visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region. The patient had undergone surgery for appendicitis when visiting for the soccer World Cup, because of pain in the right inguinal region.

Culture of lymph node tissue. The lymph node specimens were homogenized in 1 ml of brain heart infusion broth and inoculated on blood-enriched Columbia agar and in an endothelial cell culture system using locally prepared polyclonal antibodies, since species of both genera have common antigens which are responsible for cross-reactions.
able domains (VD) I and II of the *C. trachomatis* major outer membrane protein (MOMP)-encoding gene, allowing differentiation of serovars (32). The amplified DNA fragment was sequenced with the same primers, as previously described.

**RESULTS**

Pathological examination of lymph node tissue. Pathological examination of lymph node tissue revealed the presence of central abscesses containing neutrophils and necrotic debris surrounded by epithelioid cells, macrophages, and a few multinucleated giant cells. Although not specific, histological findings were compatible with either cat scratch disease or LGV.

Culture of lymph node tissue. The Gram stain performed on tissue homogenates was negative. Standard cultures and mycobacterial cultures remained sterile after 15 days and 2 months of incubation of culture media, respectively. In contrast, Gimenez staining of cell monolayers, performed on the 15th day of incubation of the shell vials, revealed the presence of intracellular bacteria. Such bacteria were also revealed by the immunofluorescence technique, using anti-*B. henselae* or anti-*Bartonella quintana* antibodies. However, after the identification of these bacteria as *C. trachomatis* by the PCR sequencing technique, subcultures performed using the same ECV304 endothelial cells allowed detection of bacterial growth within 48 h of incubation of the shell vials, which is compatible with the intracellular development cycle of this species (18).

PCR sequencing. No PCR amplification was obtained with primers OHVE1 and OHVE3 or rD1 and rP2. In contrast, the PCR assay using primers incorporating ID4 and rD1 yielded a 500-bp DNA fragment, which was sequenced. The sequence was compared, using BLAST software, with sequences deposited in GenBank and showed 100% similarity with the *C. trachomatis* 16S rRNA gene (accession number M59178). The PCR assay incorporating the primers S’-MOMP and BFP-2 allowed amplification of a 490-bp DNA fragment including *C. trachomatis* VDI and VDII. The sequence of this fragment showed 99% identity with a MOMP-derived DNA fragment previously described for *C. trachomatis* biovar LGV serovar L2 (32), with 100% homology in the VDI region, whereas variation was noted in base position 490 in VDII, where an adenine was replaced by a guanine, leading to replacement of an asparagine by an aspartic acid as amino acid 142.

Serology. Patient’s serum collected at the time of hospitalization displayed anti-*C. trachomatis* antibodies, with immunoglobulin G (IgG) and IgA titers of 1:64 and 1:16, respectively, by microimmunofluorescence. Anti-*B. henselae* antibodies were also detected in patient’s serum, with an IgG titer of 1:50 by microimmunofluorescence. Serologies for HIV, *T. pallidum*, and *F. tularensis* were negative.

**DISCUSSION**

*C. trachomatis* biovar trachoma and serovars D, E, F, G, H, I, J, and K are responsible for oculogenital infections with worldwide distributions (3). In Europe and North America, these serovars are responsible for the majority of bacterial sexually transmitted diseases. In contrast, LGV, due to *C. trachomatis* biovar LGV and serovars L1, L2, and L3, is a sexually transmitted disease restricted to India, southeastern Asia, sub-Saharan Africa, South America, and the Caribbean. Sporadic cases are diagnosed in other areas (3), including exceptional cases reported in France (26). Serovar L2 is most frequently isolated in areas where the disease is not endemic, whereas serovar L1 is uncommon (2). The disease is characterized by three clinical stages. The first stage corresponds to a primary genital lesion, usually a small papule or a herpetiform ulcer, which often remains unnoticed. LGV is most often recognized at the second stage, which corresponds to a local lymphadenopathy (most often inguinal), which may evolve to a local abscess (or bubo), which ruptures in approximately 30% of cases, and fistulize to the skin. This stage may include systemic manifestations, such as fever, headaches, and myalgia. The third stage correspond to late fistula and stricture formation.

A specific diagnosis of LGV is established by isolation of *C. trachomatis* from bubo pus in 30% of cases, or occasionally from the cervix in women or the urethra in men, and rarely from systemic sites (3). However, isolation of this pathogen remains difficult, and it can only be done in laboratories with specially equipped biohazard facilities and personnel experienced in cell culture. Only a few isolates have been obtained in the last 20 years, especially in the United States (2) and in Europe (8, 26). More recently developed molecular biology techniques, including PCR-based techniques, may be useful (9, 11). Such techniques are not used routinely for diagnosis of LGV and are not available in most areas where LGV is endemic. Thus, serology using the complement fixation test or the microimmunofluorescence technique remains the diagnostic method most frequently used (3). Antibody titers of ≥1:64 by the complement fixation test or ≥1:512 by microimmunofluorescence techniques are considered highly indicative of LGV in patients with typical clinical presentation (3) but are not fully specific because of cross-reactions among different *Chlamydia* species, biovars, and serovars.

We report a patient with second-stage LGV, a 21-year-old Romanian man infected with *C. trachomatis* biovar LGV serovar L2. The patient presented with an inguinal lymphadenopathy for which cat scratch disease was a possible etiology. Thus, lymph node specimens, as well as a serum sample, were sent to our laboratory for *Bartonella*-specific culture and serology. We used a previously described centrifugation shell vial method for isolation of *Bartonella* species (15, 21). A direct immunofluorescence assay using rabbit polyclonal anti-*Bartonella* sp. antibodies revealed fluorescent bacteria within the cell cultures. However, the use of a PCR sequencing technique led to the unexpected identification of these bacteria as *C. trachomatis* biovar LGV rather than *Bartonella* sp. Such strain identification cannot be considered misinterpretation due to cross contamination, since *C. trachomatis* biovar LGV was never sequenced before in our laboratory. A serum sample collected at the time of hospitalization revealed the presence of anti-*C. trachomatis* antibodies (IgG titer, 1:64; IgM titer, 1:16 [by microimmunofluorescence]) but also low-titer anti-*B. henselae* antibodies (IgG titer, 1:50 by microimmunofluorescence). Interestingly, our strain was obtained in endothelial cell cultures, whereas McCoy cells are usually recommended for isolation of this species from clinical specimens (3). *C. trachomatis* has been grown in vitro in McCoy cells (28, 29, 31), HeLa cells (13, 23), and BGMK cells (10, 12), including in shell vial systems (10, 29), but to our knowledge this is the first time growth in endothelial cells has been reported for diagnostic purposes. This is compatible with a recent demonstration that *Chlamydia pneumoniae* and also *C. trachomatis* can infect human umbilical vein endothelial cells in vitro (7).

Cross-reactions between *Chlamydia* spp. and *Bartonella* spp. have been previously described (4, 17) and recently characterized (17). These cross-reactions have led to misinterpretation of *Chlamydia* sp. serology in the past. *Chlamydia* species were proposed as probable etiological agents of cat scratch disease (5) because sera from many patients with cat scratch disease...
reacted with chlamydial antigens. Recent investigations have shown that *B. henselae* is the major etiologic agent of this disease (1, 22), whereas serum reactivity against *Chlamydia* antigens in fact represented cross-reactions. Only 27 *Chlamydia*-related endocarditis cases have been reported in the literature (17, 25). In one case, a specific diagnosis was established by culturing *Chlamydia psittaci* from blood cultures (27), whereas in another case, *C. pneumoniae* by culturing from a left aortic leaflet (25). For all 25 remaining cases, cross-reactions has been eliminated. Thus, the possibility of *C. pneumoniae* by culturing should be performed concomitantly, especially for clinical manifestations in which species of both *Chlamydia* serologies should be performed concomitantly, especially for clinical manifestations in which species of both proven and related to the major etiological agent.

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**REFERENCES**


