Isolation of *Rickettsia prowazekii* from Blood by Shell Vial Cell Culture

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Received 1 April 1999/Returned for modification 13 May 1999/Accepted 23 July 1999

A blood sample from a patient who returned from Algeria with a fever inoculated on human embryonic lung fibroblasts by the shell vial cell culture technique led to the recovery of *Rickettsia prowazekii*. The last clinical strain was isolated 30 years ago. Shell vial cell culture is a versatile method that could replace the classic animal and/or embryonated egg inoculation.

Epidemic typhus is a body louse-transmitted disease due to *Rickettsia prowazekii*. Recent reports of cases occurring in Burundi (12), in Russia (16), and in Peru (unpublished data) and the present case from a patient returning from Algeria must remind us that epidemic typhus is a reemerging disease. The laboratory diagnosis of epidemic typhus is based on serology but is hampered by cross-reaction with murine typhus (7). The shell vial assay (8), originally devised for the isolation of viruses, has now been adapted by members of our team for the isolation of spotted fever group rickettsiae (7). In this report, we describe the first application of this method to the isolation of *R. prowazekii*.

**Case report.** A 65-year-old man was referred to our hospital center in October 1998 for fever and diarrhea. This native Algerian, who usually lives in France, returned to France by boat after a visit to Algeria. On arrival in Marseille, he suffered fever, vomiting, myalgias, and diarrhea. On examination he presented with a high fever of 40.6°C and a dissociated pulse. The patient exhibited mild confusion. A discrete rash and splenomegaly were noticed. A presumptive diagnosis of typhoid fever was made, and treatment with ceftriaxone (3 g/day, administered intravenously) was started immediately. By day three, blood and stool cultures were negative and the patient’s condition had worsened. He was still febrile, dyspnea was noted, the rash became purpuric, and the patient was semicomatose. A diagnosis of typhus (murine or epidemic) was suspected and doxycycline (200 mg/day) was prescribed. His condition rapidly improved, as he was afebrile within 3 days.

A blood sample from the patient was inoculated onto three shell vials containing human embryonic lung (HEL) fibroblasts grown on coverslips, as previously described (6), in a biosafety level 3 containment laboratory. Detection of growing bacteria was carried out by cytocentrifugation of 100 μl of one shell vial supernatant for further Gimenez staining and directly inside the shell vial by immunofluorescence. After fixation with cold acetone, the vial was washed twice with phosphate-buffered saline (PBS). One hundred microliters of the patient’s convalescent serum (taken 1 week after admission), diluted 1:50 in PBS with 3% nonfat dry milk, was added, and the vial was incubated at 37°C with 100 μl of a fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin (Ig) (Fluoline H; Biomerieux, Marcy l’Etoile, France) diluted 1:200 in PBS containing 0.02% Evans blue. After three washes with PBS, the coverslip was mounted (cells face down) in phosphate-buffered glycerol medium (pH 8) and examined at 400× with a Zeiss epifluorescence microscope. This detection procedure was performed on days 7 and 14. The supernatant of a positive shell vial was used for PCR-based identification and was inoculated on confluent layers of HEL cells in a 150-cm² culture flask in order to establish the isolate.

DNA extracts suitable for use as the template in PCR assays were prepared from one remaining shell vial. These DNA extracts were amplified by using PCR incorporating primers that allow amplification of genes encoding the citrate synthase (*glcA*) and the rickettsial outer membrane protein rOmpB (*ompB*). Sequencing reactions were carried out by incorporating the same primers as those used for amplification, and sequence products were resolved in the ABI PRISM 377 automatic sequencing system (Perkin-Elmer Applied Biosystems, Warrington, United Kingdom).

As this patient came from an area not known as a region of endemicity for epidemic typhus, determination of antibody to *R. typhi* was initially performed by indirect fluorescent-antibody assay, as previously described (12), on sera taken on admission and 1 week later. Antibodies to *R. prowazekii* were further determined.

Gimenez staining performed on day 7 was negative. Gimenez staining performed on day 14 yielded numerous red-stained bacteria located in the cytoplasm of HEL cells. The coverslip of the same shell vial allowed detection of numerous fluorescent bacteria in intracellular locations (Fig. 1). The supernatants of positive shell vials inoculated on confluent layers of HEL cells allowed establishment of the isolate. The nucleotide sequence obtained was compared to all previously reported sequences of these genes by a Gapped Blast 2.0 (National Center for Biotechnology Information) search of the GenBank database. The sequences derived from the shell vial isolate were found to share 100% sequence similarity with those of *R. prowazekii* already deposited in GenBank. By indirect fluorescent-antibody assay seroconversion to *R. typhi* was first demonstrated (in convalescent serum, an IgG titer of 1:2,048 and an IgM titer of 1:128 were determined). In convalescent serum, an IgG antibody titer of 1:4,096 and an IgM antibody titer of 1:128 against *R. prowazekii* were determined.

The clinical isolation of *R. prowazekii*, based on inoculation of clinical samples on animals, has not been reported for 30
We have used this technique routinely and successfully to isolate viruses as well as facultative or strictly intracellular bacteria. A versatile and rapid method which can be applied to many clinical samples (10). The centrifugation-shell vial system (8) is designed to obtain an isolate. Furthermore, they are easily contaminated.

An animal supplier and often need several blind passages to obtain animals, and so may help save animals, and is well adapted to isolation of such bacteria in developed countries for travelers returning from areas of endemicity. Furthermore, as shown in this case, it allows determination of the infecting bacterial species, whereas sera cross-react extensively with closely related species.

As the use of the shell vial technique for isolation of rickettsiae is restricted to specialized research and public health laboratories that have biosafety level 3 containment, it is not suitable for use in most clinical microbiology laboratories, especially in poor countries where epidemic typhus is more likely to reemerge. However, in its favor, this procedure provides a means for the isolation of a wide range of intracellular bacteria that are usually isolated by inoculation of animals, and so may help save animals, and is well adapted to isolation of such bacteria in developed countries for travelers returning from areas of endemicity. Furthermore, as shown in this case, it allows determination of the infecting bacterial species, whereas sera cross-react extensively with closely related species.

REFERENCES


