Intraerythrocytic Presence of *Bartonella henselae*

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Received 21 November 1994/Returned for modification 3 January 1995/Accepted 1 March 1995

Recent reports in the medical literature emphasize the risk of zoonotic disease and the high degree of prevalence of asymptomatic feline infection with *Bartonella (Rochalimaea) henselae*. While investigating *Bartonella* bacteremia in cats, we used transmission electron microscopy to demonstrate *B. henselae* in the erythrocytes of persistently bacteremic cats.

*Bartonella* species (emended to include those organisms previously classified as *Rochalimaea* species) have been reported to cause cat scratch disease, bacillary angiomatosis, Carrion’s disease, endocarditis, Trench fever, and fever of unknown origin (3). *Bartonella henselae* appears to be the predominant cause of cat scratch disease in people. *Bartonella bacilliformis* (the agent of Carrion’s disease) has been reported to reside within erythrocytes (1). However, other species of *Bartonella* (*B. quintana*, *B. vinsonii*) and the more distantly related feline blood parasite, *Hemobartonella felis*, are described as maintaining an epicyclic location adjacent to the erythrocyte (13).

*Bartonella* organisms are difficult to isolate by conventional blood culture. However, by using the lysis centrifugation method, the bacteria can be isolated from whole blood on artificial medium supplemented with blood or hemin to satisfy the hemin requirement of *Bartonella* species (11). The culture technique involves drawing blood into a collection tube containing a lysing solution of sodium dodecyl sulfate–saponin–polypropylene glycol (Isolator; Wampole Laboratories, Cranbury, N.J.). Following centrifugation and removal of the supernatant, the lysed erythrocyte concentrate is applied to blood agar plates and incubated in a CO2-enriched environment at 37°C. Cultivation of *B. henselae* is enhanced by cellular lysis or the addition of hemin for growth. These observations suggest an intimate relationship with or residence within the erythrocyte.

Blood was aseptically obtained by jugular venipuncture from two cats with suspected *B. henselae* bacteremia. Persistent *B. henselae* bacteremia was subsequently documented by obtaining positive blood cultures at 1- to 3-month intervals for approximately 1 year. At the time of the initial isolation, the species of the organisms were determined by 16S rRNA gene sequencing and restriction endonuclease digestion of chromosomal DNA. Identical morphologic and growth characteristics provided phenotypic identifications of subsequent isolates from each cat. When the original lysis centrifugation culture from cat A and a second culture from cat B were performed, one drop of whole blood from each cat was also immediately placed into McDowell and Trump’s 4F:1G fixative and was processed for transmission electron microscopy (5).

Electron micrographs revealed organisms within erythrocytes (Fig. 1). Occasional erythrocytes contained multiple organisms that were transsected as well as cut longitudinally within the same cell. Electron microscopic examination of 1,220 erythrocytes from cat A identified 35 (2.9%) infected cells, while 40 of 645 (6.2%) erythrocytes from cat B contained intracellular *B. henselae*. No epicyclic or extracellular organisms were observed in samples derived from either cat. Although they were potentially altered during processing, failure to identify any epicyclic organisms from the electron micrographs of either cat supports an intraerythrocytic presence for *B. henselae*. The sizes and morphologies of these intraerythrocytic bacteria were consistent with our earlier transmission electron microscopic observations of *Bartonella* organisms cultured on blood agar and were not dissimilar to photomicrographs of *B. bacilliformis* published elsewhere (2, 4).

The intraerythrocytic presence of *B. henselae* is not surprising. The mechanism of entry is unknown. In Fig. 2, it appears that the erythrocyte membrane is engulfing the bacteria. Because erythrocytes are not normally capable of endocytosis, we suspect a bacterium-induced or forced endocytosis. *Salmonella typhimurium* enters cells assisted by the rotation of its flagella (7). By using specialized staining techniques, flagella are clearly present on *B. bacilliformis*. The flagella probably perform an important role in cellular entry. Flagella have not yet been observed on *B. henselae*, however. Invasion studies with *B. bacilliformis* have demonstrated decreased erythrocyte penetration following bacterial exposure to antiflagellin antibody (12). Figure 3 illustrates the existence of a pore between the bacterium and the extracellular fluid space. This communication with the surrounding plasma, identified in electron micrographs from both cats, may represent a nutrient channel or perhaps a route of entry or egress for the bacteria into or from the cell.

*B. bacilliformis* also reportedly secretes a protein termed deformation factor which deforms the erythrocyte membrane and presumably aids in the subsequent binding and entry of the bacteria (10). To date, deformation factor has not been reported in association with *B. henselae* or any *Bartonella* species other than *B. bacilliformis*.

Traditionally, cat scratch disease has been considered a self-limiting illness, and patients are infrequently treated with antibiotics. *B. henselae* is susceptible to several antimicrobial agents in vitro; however, in vivo infection in humans has been quite refractory to antimicrobial treatment (8, 9). The inaccessibility of many routinely prescribed antibiotics to the intraerythrocytic bacteria, as observed in the electron micrographs presented here, provides a potential explanation for poor antimicrobial efficacy.

Although the clinical course of cat scratch disease is typically mild, disease manifestations in immunocompromised and occasional immunocompetent individuals can be severe (6).
Therefore, interest in Bartonella vaccine development is widespread. Knowledge of the habitat of the bacteria and its interaction with the host’s immune system is necessary to achieve success in this endeavor and to attempt to answer other questions that currently elude us regarding members of the genus Bartonella. The demonstration of B. henselae in erythrocytes will, it is hoped, further these efforts.

We acknowledge the Electron Microscopy Laboratory at North Carolina State University College of Veterinary Medicine for the preparation of the electron micrographs.

REFERENCES

FIG. 1. Electron photomicrograph of two B. henselae organisms inside a feline erythrocyte. Staining was with methanol uranyl acetate and then lead citrate. Magnification, ×16,900.

FIG. 2. Intraerythrocytic B. henselae illustrating possible engulfment by erythrocyte membrane (arrow). Staining was with methanol uranyl acetate and then lead citrate. Magnification, ×21,000.

FIG. 3. Electron photomicrograph of intraerythrocytic B. henselae illustrating the existence of a pore between the bacterium and the extracellular fluid space (arrow). Staining was with methanol uranyl acetate and then lead citrate. Magnification, ×38,000.