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Bartonella henselae Infects Human Erythrocytes

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ABSTRACT *Bartonella henselae*, a facultative intracellular bacterium, has been known as the agent of cat scratch disease, bacillary angiomatosis, peliosis hepatis, endocarditis, and bacteremic syndrome in humans. *Bartonella* species can cause intraerythrocytic infections and have been isolated from the bloodstream of patients by several methods. It was demonstrated that *B. bacilliformis* and *B. quintana* infect human endothelial cells and human erythrocytes and *B. henselae* infects erythrocytes of cats. The aim of this study was to investigate through transmission electron microscopy whether *B. henselae* infects mature human erythrocytes. One red blood cell (RBC) unit received an experimentally standard strain of *B. henselae*. Blood aliquots were collected from the infected unit immediately after inoculation, at 30 min and 1, 5, 10, and 72 h for ultrastructural evaluation. *B. henselae* was seen adhering to human erythrocytes 10 h after inoculation and inside the erythrocyte after 72 h. This study demonstrates that *B. henselae* adheres to and invades mature human erythrocytes. The results favor the possibility that erythrocytes can serve as a primary target in *Bartonella* spp. infections. From this observation, further studies are warranted to prevent *Bartonella* spp. transfusional transmission.

KEYWORDS *Bartonella henselae*, electron microscopy, erythrocytes

Bacteria of the genus *Bartonella* are fastidious, gram-negative, aerobic bacilli that comprise numerous species [1]. The extreme diversity of disease manifestations is dependent on the infecting of *Bartonella* spp. and on the immune status of the patient [2].

Three *Bartonella* species are associated with an increasing number of clinical manifestations in human beings. *B. bacilliformis* causes Oroya fever and verruga peruana; *B. henselae* causes cat-scratch disease, bacillary angiomatosis, peliosis hepatis, endocarditis, and septicemia; and *B. quintana* causes trench fever, bacillary angiomatosis, bacteremia and endocarditis [3, 4].

Erythema nodosum, granuloma annulare, erythema multiforme, exanthemas, thrombocytopenic purpura, chronic adenopathy, and chronic fatigue syndrome have been associated with *Bartonella* spp. infection [4, 5].

Each *Bartonella* species appears to be highly adapted to one or few mammalian reservoir hosts, in which *Bartonella* causes a long-lasting intra

erythrocytic bacteremia as a hallmark of infection [6, 7]. The bacteria can persist in the bloodstream of the host as the result of intraerythrocytic parasitism [2, 8].

The primary intracellular niche in *Bartonella* sp. infections remains unclear [9].

Intraerythrocytic localization of *B. henselae* has been demonstrated in cat erythrocytes and *B. bacilliformis* have been observed within erythrocytes during the acute phase of Carrion disease (Oroya fever) [10, 11]. Trench fever and "modern-day trench fever" are a manifestation of infection with *B. quintana* that is observed in the erythrocytes of bacteremic homeless people [2, 4, 12]. *Bartonella* sp. has a tropism for endothelial cells, and intracellular *B. henselae* can be identified in endothelial cells infected in vitro [7]. Recently, it has been shown that *B. tribocorum* occurs in the erythrocytes of rats and does not cause hemolysis [6, 8].

In the present report, our objective was to demonstrate that *B. henselae* adheres to and invades mature human erythrocytes.

STUDY DESIGN

The *B. henselae* strain (Houston 1, American Type Culture Collection, Rockville, MD, ATCC 49882^T) used in this study was supplied by the bacteria bank of Adolpho Lutz Institute, São Paulo, SP, Brazil. The bacteria was grown on 5% sheep blood agar plates and incubated at 37°C in 5% CO_2-enriched atmosphere.

Bacterial suspension was performed by mixing bacteria colonies obtained from sheep blood agar plates with brain and heart infusion (BHI). This suspension of *B. henselae* colonies was used to obtain equivalence with tube 10 of the McFarland scale, which determined an initial suspension with approximately 3×10^9 colony-forming units (CFU)/mL[13].

One red blood cell (RBC) unit from healthy blood donor was collected in CPDA1 (Fresenius, Asem-NPBI, Itapecerica da Serra, São Paulo, Brazil). Blood was centrifuged (3500 g, 10 min) for component preparation and no additive/preservative solution was added. A total of 9 mL of RBC was collected using a sterile connecting sampling site coupler (Baxter Healthcare Corporation, Deerfield, USA) and was infected with 1 mL of the bacterium suspension. Then, 50 µL of RBC was collected at minute 0 (immediately after infection) and the remaining of the infected RBC was kept incubated at 37°C. Five other aliquots were collected at minute 30 and hours 1, 5, 10, and 72 for electron microscopy evaluation, after conservation in Karnovisky medium [13].

Briefly, the samples were then fixed in 2% osmium tetraoxide for 2 h and left overnight in uranile acetate 0.5%. Dehydration was performed with acetone solutions; inclusion was made in epoxy resin, which was heated to 60°C for 48 h. The semithin sections were made around 300 nm with a glass razor in the MT-6000 XL-RMC ultramicrotome and stained with toluidine blue 2% for better area selection. Ultrathin sections of approximately 90 nm were obtained with a diamond razor (Diatome), with the Leica Ultracut UCT ultramicrotome, laid on copper mesh, stained with lead citrate, and examined in the Zeiss LEO-906 electron microscope.

RESULTS AND DISCUSSION

Bartonella typical structures could be seen adhering to human erythrocytes in samples collected 10 h after infection (Figure 1) and inside erythrocytes after 72 h (Figure 2). They presented the trilaminar

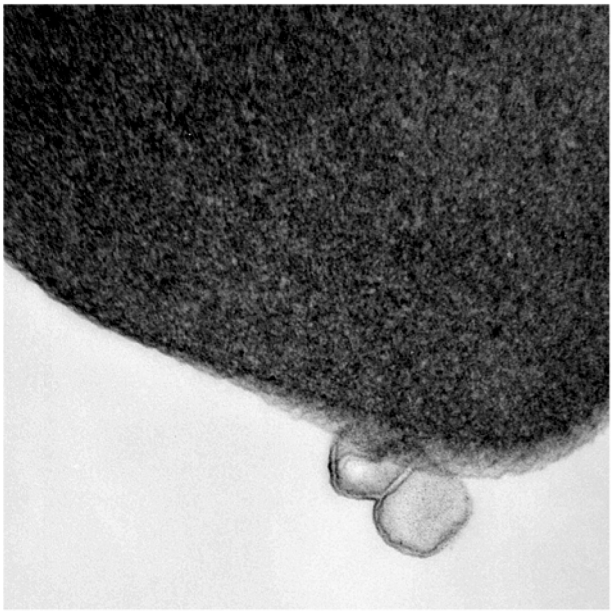

FIGURE 1 **Adherence of *B. henselae* in human erythrocytes. Samples of infected RBC were analyzed by transmission electron microscopy (TEM). *B. henselae* could be observed adhering to human erythrocytes in samples collected 10 h after infection. TEM analysis demonstrates the typical trilaminar wall bacilli (original magnification, ×20,000).**

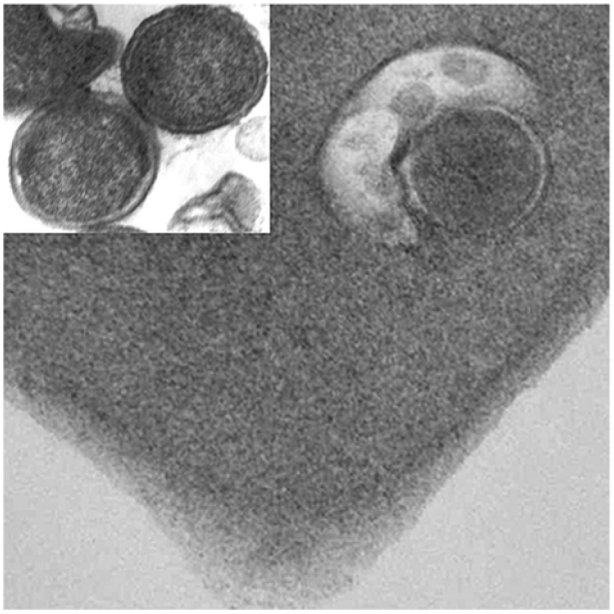

FIGURE 2 Invasion of human red blood cell (RBC) with *B. henselae*. Erythrocytes were infected with *B. henselae* strain ATCC 49882. Transmission electron microscopy (TEM) analysis showed intracellular *B. henselae* inside an erythrocyte 72 h after infection (original magnification, ×35,970). In detail, TEM photograpy of the original culture of *B. henselae* used in infection studies.

wall, which has been previously shown to be specific of gram-negative bacteria [13, 14].

Bartonella species are closely associated with erythrocytes in their natural hosts. There is evidence that *B. henselae* can infect endothelial cells [15, 16, 17], epithelial cells [18, 19], and monocytes or macrophages [20, 21], but there is controversiy about its capability to invade red blood cells [22–25].

Species of *Bartonella* can live inside red blood cells, as was demonstrated for *B. quintana* and *B. bacilliformis*. Intraerythrocytic localization of *B. henselae* has been demonstrated in cat erythrocytes [7, 10, 12].

Previous studies have shown that *B. henselae* can infect freshly isolated human CD34 hematopoietic progenitor cells but not human erythrocytes [22].

Man is a reservoir of *Bartonella* sp. and the risk for blood transmission should be considered. In a previous study at the State University of Campinas (UNICAMP) it was demonstrated that *B. henselae* remains viable in red blood cell units after standard storage period (data not shown). These data reinforce the possibility of infection through blood units collected from asymptomatic blood carriers. Furthermore, the tests that are usually carried out for triage of donated blood bags do not detect these agents, including the routine hemoculture.

Human erythrocytes were infected with *B. henselae* and invasion of erythrocytes was demonstrated by TEM. In conclusion, our study favors the possibility that erythrocytes can serve as the primary target in *Bartonella* spp. infections. From this observation, further studies are warranted to evaluate the potential of *Bartonella* sp. transfusional transmission.

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