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Failure to detect *Bartonella henselae* infection in synovial fluid from sufferers of chronic arthritis

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**Abstract** *Bartonella henselae* causes granulomatous and indolent infection in the immune competent human, and angioproliferation in the context of persistent infection and impaired immunity. This bacterium is found in up to 40% of household cats, from which humans acquire it by either a cat scratch or a bite (hence the name, cat-scratch disease). Approximately 5% of Australian and US blood donors have serological evidence of past infection, but most associated illnesses are mild or subclinical. A number of lines of evidence prompted us to consider a relationship between rheumatoid arthritis (RA) and *Bartonella* infection. These include epidemiological associations with household pet exposure; apparent responsiveness of some RA cases to tetracycline therapy; the granulomatous and angioproliferative nature of *Bartonella* lesions; the insidiousness and high seroprevalence of this infection in the community; and even reported *Bartonella* infection mimicking juvenile RA. In a small group of patients with chronic arthritides, we found no direct evidence of humoral antibodies to, nor of persistent infection with, *Bartonella henselae* in synovial fluid. While larger and more invasive studies are likely to provide more confident exclusions of this hypothesis, this suggests that persistent *Bartonella* infection is unlikely to play a major role in RA.

**Key words** Rheumatoid arthritis · Aetiology · *Bartonella henselae* · Infection · Persistence

**Introduction**

There is increased interest in the search for infective triggers of autoimmune diseases, and demonstrations of the value of tetracycline therapy in early rheumatoid arthritis (RA) [1] highlights this interesting possibility. Aggressive inflammatory responses associated with mononuclear infiltrates, granulomatous change and angioproliferation may also be seen in certain bacterial infections, most strikingly in those caused by a newly recognised fastidious intracellular Gram-negative bacillus, *Bartonella henselae* (against which tetracycline therapy is effective) [2]. The best-described clinical syndrome resulting from this infection in humans, cat-scratch disease (CSD), typically presents as a benign unilateral lymphadenitis involving lymph nodes draining a wound or granuloma following a cat-scratch or bite [3, 4]. Persistent *Bartonella* infection in humans results in the production of granulomatous and pseudo-neoplastic angioproliferative lesions in skin (bacillary angiomatisis) and deep tissues (bacillary peliosis), which respond to antimicrobial therapy [5]. This phenomenon is typical of *B. henselae* infection in the immune compromised [5] as well as in the late stages of *B. bacilliformis* infection [6].

Up to 40% of domestic cats in the USA and Australia have *B. henselae* circulating in their blood, and represent a huge reservoir of infection. *B. henselae* is transmitted between domestic cats by the common cat flea and feline bartonellosis is directly related to flea burdens and environmental conditions [3, 7]. There has been no demonstration to date of a particular subset of this species, which is more virulent for humans, although there is some considerable diversity among clinical human and feline isolates [8]. The rate of clinical human infection is estimated to be approximately 1 in 10,000 annually in the USA, and results in thousands of hospitalisations each year [9]. Difficulty in diagnosis, high seroprevalence in the general population (ca. 5% of blood donors) [9], and reports of *B. henselae* as a common cause of prolonged fever of unknown origin (FUO) in children [10] suggest that this disease is significantly underdiagnosed, including within Australia [4].

The risk of development of RA may be increased by animal exposure (including cats) in the 5 years prior to the onset of the disease, and an association with cats in the prepubertal period correlates significantly (odds
ratio 4.9) with the subsequent development of RA [11, 12]. Bartonella infection has been reported to mimic juvenile chronic arthritis [13] and may be associated with reactive arthritis in adults [14]. A specific amino acid sequence in the HLA-DR (MHC class II) epitope is a marker for genetically susceptible individuals [15], and may provide a link to the increased risk associated with cat exposure [16]. Such associations, along with the granulomatus and angioproliferative nature of the pathological lesions of bartonellosis, led us to investigate a possible link between RA and Bartonella infection.

Materials and methods

Synovial fluid (SF) samples were collected from patients presenting to rheumatologists with RA and non-RA arthropathies as part of the routine treatment of the patient (see Table 1).

Culture

Freshly aspirated SF (0.5 ml) was inoculated into sterile Brucella broth, onto chocolate agar and into specialised liquid growth medium [17], both before and after freezing (the latter manoeuvre having been shown to increase yield [18]). Cultures were incubated in humidified 5% CO₂ at 37 °C for 21 days and examined for growth at regular intervals. Strains utilised for DNA controls and infecting cell lines for serological examination were from the American Type Culture Collection (ATCC): ATCC 49882 (“Houston-I” type strain) and ATCC 49793.

Nucleic acid amplification

Control B. henselae strain ATCC 49882 was grown in 5% CO₂ on chocolate agar plates for 5 days at 37 °C to serve as a control. A range of dilutions were prepared in phosphate-buffered saline (PBS) for use in polymerase chain reaction (PCR), both in crude form and after DNA extraction (Wizard Genomic DNA Purification Kit, Cat. #A1120, Promega, USA; per manufacturers instructions). An established protocol for PCR of B. henselae [9] using AmpliTaq (Cetus Perkin-Elmer) 1 U/50 μl reaction was modified as follows: denaturation at 94 °C for 5 min; then cycle 2: 94 °C, 30 s; 56 °C, 1 min; 72 °C, 45 s (35 cycles); cycle 3: 72 °C, 10 min (one cycle). Samples were electrophoresed in a 1% agarose gel and visualised with ethidium bromide under UV light, as previously described [19].

Freeze/thawed bacteria were used to spike SF samples in final concentrations of 108, 103, 10, 1 and 0.1 colony forming units (CFU)/ml and tested with and without prior DNA extraction. The DNA extracted from 1.0 ml of each sample was resuspended in 100 μl. Following DNA extraction, 35 μl of each sample was used as a template in a 50-μl PCR.

To determine the sensitivity of the PCR to detect DNA in SF without DNA extraction, samples were spiked with purified B. henselae ATCC 49882 (Houston-I) DNA to final concentrations of 180, 18, 1.8 and 0.18 ng/μl DNA. Five microlitres of each spiked suspension was used as a template in a 50-μl PCR. As a control, SF spiked with purified DNA to a final concentration of 9 × 10⁻⁷ ng/μl was then extracted and reconstituted at tenfold concentration after extraction; 35 μl was then used in a 50-μl PCR.

Indirect fluorescent antibody test

IFATs were conducted on each of the SF samples as previously described [20]. Briefly, HEP-2 cells, which were infected with B. henselae ATCC 49793 for 10 days, were harvested, washed and acetone-fixed onto 18-well glass slides. The SF samples were diluted from 1:10 to 1:2048 for use on these slides. A human clinical sample with a predetermined titer of 1:2048 served as a positive control. Following incubation of cells with SF and washing, fluorescein isothiocyanate (FITC)-conjugated antihuman antibody was added and slides were observed under a fluorescence microscope.

Results

Twenty different SF samples were collected (see Table 1). Adult patients (n = 18) suffered from RA (n = 11), osteoarthritis (n = 4), psoriatic arthritis (n = 2) and Crohn’s disease (n = 1). The adult group had a mean age of 59.7 years (range 30–82 years) and had suffered from arthritis for an average of 5.7 years (range 1–18 years). The male:female ratio was 8:10. Aspirates were also obtained from two 6-year-old girls with a diagnoses of juvenile chronic arthritis of 6 months and 1 year durations, respectively.

No bacterial colonies were observed on any of the chocolate agar plates (inoculated with either neat or pelleted fluid samples). No B. henselae growth was detected in automated broth culture (Bactec 9240) or on directly inoculated chocolate plates, nor in specialised broth culture as described above.

Sensitivity of the PCR assay

PCR conducted on freeze-thawed, whole bacterial suspensions in PBS gave a threshold for detection of 1 × 10⁻³ CFU/ml in the PCR assay, reflecting a threshold of 0.1 CFU/ml in the original sample (using 10 μl in a 50-μl PCR). When freeze-thawed bacteria were used to spike SF, a final concentration of 0.5 CFU/ml, and not 0.05 CFU/ml, in the original SF sample was...