The *Bartonella henselae* sucB gene encodes a dihydrolipoamide succinyltransferase protein reactive with sera from patients with cat-scratch disease

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*Bartonella henselae* is a recently recognized pathogenic bacterium associated with cat-scratch disease, bacillary angiomatosis and bacillary peliosis. A recombinant clone expressing an immunoreactive antigen of *B. henselae* was isolated by screening a genomic DNA cosmid library by Western blotting with sera pooled from patients positive for *B. henselae* IgG antibodies by indirect immunofluorescence (IFA). The deduced amino acid sequence of the 43.7 kDa encoded protein was found to be 76.3% identical to the dihydrolipoamide succinyltransferase enzyme (SucB) of *Brucella melitensis*. SucB has been shown to be an immunogenic protein during infections by *Brucella melitensis*, *Coxiella burnetii* and *Bartonella vinsonii*. The agreement between reactivity with a recombinant SucB fusion protein on immunoblot analysis and the results obtained by IFA was 55% for IFA-positive sera and 88% for IFA-negative sera. Cross-reactivity was observed with sera from patients with antibodies against *Brucella melitensis*, *Mycoplasma pneumoniae*, *Francisella tularensis*, *Coxiella burnetii* and *Rickettsia typhi*.

INTRODUCTION

*Bartonella henselae* is a recently recognized pathogenic bacterium that has been found to be associated with cat-scratch disease (CSD), bacillary angiomatosis and bacillary peliosis (Cockerell & Friedman-Kien, 1988; Dolan et al., 1993; Koehler et al., 1994; Reed et al., 1992; Zangwill et al., 1993). Of these syndromes, CSD is the most common and affects an estimated 25 000 people in the United States annually, the majority of whom are children (Jackson et al., 1993). Severe and protracted cases of CSD often require extensive clinical and laboratory investigation, including lymph node biopsy. The use of serological tests specific for *B. henselae* may help in avoiding invasive procedures in these patients.

Current diagnostic methods for determining the presence of *Bartonella* antibodies in CSD include indirect immunofluorescence assay (IFA) and enzyme immunoassay (EIA). Sensitivity of the IFA has been reported to vary from 32 (Bergmans et al., 1997) to 100% (Sander et al., 1998) in different studies. A high degree of cross-reactivity of *Bartonella* serology with other intracellular pathogens has been described and has made the serologically based diagnosis of CSD problematic (La Scola & Raoult, 1996; Maurin et al., 1997; McGill et al., 1998; Sander et al., 1998). Highly purified and cloned antigens have been shown to be very sensitive and specific in serologic assays. Anderson et al. (1995) found 92% agreement with IFA-positive sera using the cloned 17 kDa antigen of *B. henselae* in an EIA. Therefore, the characterization and cloning of the individual antigens involved in eliciting an immune response to *B. henselae* in humans should aid in the development of more sensitive and specific diagnostic serologic tests.

Relatively little is known regarding the immunoreactive proteins associated with *B. henselae* infections. Only two immunoreactive *B. henselae* antigens have been cloned and characterized to date: the 17 kDa antigen (Anderson et al., 1995) and HtrA stress response protein (Anderson et al., 1996). Recently, the 45 kDa dihydrolipoamide succinyltransferase protein (SucB) was found to be immunogenic in a mouse experimentally infected with *Bartonella* (Gilmore et al., 2003). The present study was initiated to identify and...
characterize immunogenic proteins of *B. henselae* that are expressed during human infection. In this paper we describe the cloning and characterization of the gene encoding the immunoreactive dihydrolipoamide succinyltransferase enzyme (*sucB*) of *B. henselae*. Studies were also performed to evaluate the possible role for this protein as a diagnostic reagent for the serological evaluation of patients with suspected CSD.

**METHODS**

**Bacterial strains and media.** *B. henselae* strain ATCC 49793 was obtained from the American Type Culture Collections (ATCC). The *Escherichia coli* strain used was XL-1 Blue MR (Stratagene). *E. coli* strains were routinely grown in Luria–Bertani broth (LB). Kanamycin (50 μg ml⁻¹) and ampicillin (100 μg ml⁻¹) were added as appropriate. *B. henselae* was grown on freshly prepared Columbia 5% sheep blood agar plates for 7 days at 35 °C under 5% carbon dioxide. All strains were maintained at −70 °C in LB media containing 15% glycerol.

**Clinical samples.** The serum used for initially screening the *B. henselae* cosmid library was pooled from 68 cases of suspected CSD that were submitted to ARUP laboratories for confirmative diagnosis. These sera had IgG titres ranging from 1:512 to 1:4096 for *B. henselae*, as determined by IFA. The IFA titre of the pooled specimen was 1:1024. One hundred serum samples positive for IgG antibodies by IFA (titres of 1:128–1:4096), and 50 samples negative for both IgM and IgG (titres < 1:16 for IgM and < 1:64 for IgG) and 100 random healthy donors in the Salt Lake City, Utah area were used in determining the agreement, sensitivity and specificity compared to IFA. The procedures followed were in accord with the ethical standards established by the University of Utah and are in accord with the Helsinki Declaration of 1975. Specimens were collected under approval by the University of Utah Institutional Review Board (IRB#11343). Specimens were stored at −20 °C until testing commenced and were then stored at 2–8 °C while the evaluations were performed.

**Sera used for cross-reactivity analysis.** A panel of sera from patients with IgG antibodies against *Brucella melitensis* (≥1 index value (IV) is positive), *Chlamydia pneumoniae* (titre ≥ 1:64), Coxella burnetti (titre ≥ 1:16), *Mycoplasma pneumoniae* (≥0.95 U l⁻¹), *Trepconema pallidum* (titre ≥ 1:5), *Ehrlichia chaffensis* (titre ≥ 1:16), *Francisella tularensis* (titre ≥ 1:80), *Rickettsia typhi* (titre ≥ 1:256), *Rickettsia rickettsii* (titre ≥ 1:1 IV), *Leptospira* spp. (titre ≥ 1:100) and *Legionella pneumophila* (titre ≥ 1:128) was used in cross-reactivity studies. Serum testing for antibodies against *M. pneumoniae* was performed using an ELISA (GenBio); for *Brucella melitensis* using an ELISA (PANBIO); for *Chlamydia pneumoniae* using a microimmunofluorescent assay (Focus Technologies); for Coxella burnetti using an IFA (Focus Technologies); for *T. pallidum* using an IFA (SCIMEDX Corporation); for *Ehrlichia chaffensis* using an IFA (PANBIO); for *F. tularensis* using agglutination (Germaine Laboratories); for *Rickettsia typhi* using an IFA (Focus Technologies); for *Rickettsia rickettsii* using an ELISA (PANBIO); for *Leptospira* spp. using indirect haemagglutination (Focus Technologies) and for *Legionella pneumophila* using an IFA (MARDX). A single positive representative sample was used for each organism. All serum samples tested by the SucB immunoblot were also tested for *M. pneumoniae* antibodies (except for three samples in which there was insufficient volume) to assess cross-reactivity with the SucB antigen. All tests were performed according to manufacturers’ recommendation.

**Construction of *B. henselae* cosmid library.** A cosmid library of *B. henselae* ATCC 49793 was constructed using SuperCos1 cosmid vector kit (Stratagene).

**IFA.** *B. henselae*-coated slides were obtained from Focus Technologies. Serum dilutions of 1:64 were applied to each well of the IFA slide for initial testing and titrated to end-point for initial positives. The slides were incubated at 37 °C in a moist chamber for 30 min. The slides were then rinsed with PBS and soaked for an additional 5 min in PBS. The slides were allowed to air dry before fluorescein-labelled goat anti-human IgG conjugate (Sigma) was added to the slides. The slides were then incubated for 30 min and washed and dried as before. The reacted slides were read at a magnification of 400×. Samples giving 2+ or greater fluorescence were considered positive at the screening dilution. Twofold serial dilutions of the sample were performed to a 2+ fluorescence endpoint. Patient samples were considered equivocal at a dilution of 1:64–1:128; 1:256 and above was considered positive.

**Western blot.** Five microlitres whole-cell protein (6.5 mg ml⁻¹) was loaded in each well of an SDS 4–15% PAGE gel. Twenty-five microlitres protein were used in loading two-well Ready Gel preparative gels (BioRad). Electrophoresis was carried out at 200 V for 30–60 min. The separated proteins were then transferred to a nitrocellulose membrane at 100 V for 1 h using the Mini-Trans-Blot electrophoretic transfer cell (Bio-Rad). The membranes were then blocked overnight at 4 °C with 3% dry milk in TBS solution. Serum samples were diluted 1:200 in a diluent/wash solution (3% dry milk in TBS).

The sera used in this study for screening the cosmid library were preadsorbed with *E. coli* antigens. Fresh overnight cultures of *E. coli* XL-1 Blue MR containing SuperCos1 vector were pelleted by centrifugation. The supernatant was discarded, the pellet was washed with 0.92 M Tris/HCl, and resuspended in 500 μl protoplasting buffer (150 mM Tris/HCl, pH 8.0; 100 M sucrose; 8 mM EDTA). Next, 10 μl 2 mg lysozyme ml⁻¹ was added, and the cells were incubated at room temperature for 15 min. The diluted primary antibody solution was incubated with a 1:10 (v/v) dilution of the lysate for 30 min with shaking at 37 °C.

After preadsorption, the sera were reacted with the membrane containing the protein samples for 3 h on a rocking platform. The membrane was then washed three times, changing the diluent/wash every 5 min. A 1:5000 dilution of alkaline phosphatase goat anti-human IgG (γ-chain specific) conjugate (Sigma), was added to the membranes. The membrane was then incubated for 1 h and washed as before, before the addition of 5-bromo-4-chloro-3-indolyl phosphate/nitro blue (tetrazolium (BCIP/NBT) substrate (Sigma). This reaction was carried out for 5–10 min and then stopped by the addition of distilled water.

**Construction of *B. henselae* genomic library.** A genomic library of *B. henselae* ATCC 49793 was constructed using *B. henselae* genomic DNA was partially digested with BamH1. The digested DNA was sized to yield 30–40 kb fragments, dephosphorylated and then ligated into the BamH1 site of SuperCos1, previously linearized with XbaI. Packaging of the cosmids into phage and their subsequent infection in the *E. coli* strain XL-1 Blue MR were performed as described by the manufacturer.

**Preparation and analysis of whole-cell proteins.** Whole-cell proteins were prepared from *E. coli* cells grown to late exponential phase in LB medium. Whole-cell proteins were harvested by centrifugation and resuspended in 0.2 M Tris/HCl (pH 8.0). The cells were centrifuged again and resuspended in 2% Laemmli’s sample buffer, boiled for 10 min and centrifuged again. Protein concentrations were determined by the Bradford method (1976) with Coomassie protein assay reagent (Pierce). Whole-cell proteins were prepared from *B. henselae* as described above except that cells were harvested directly from Columbia sheep blood agar plates after 3 days growth. The proteins were separated on SDS 4–15% PAGE and were stained with Coomassie blue.

After preadsorption, the sera were reacted with the membrane containing the protein samples for 3 h on a rocking platform. The membrane was then washed three times, changing the diluent/wash every 5 min. A 1:5000 dilution of alkaline phosphatase goat anti-human IgG (γ-chain specific) conjugate (Sigma), was added to the membranes. The membrane was then incubated for 1 h and washed as before, before the addition of 5-bromo-4-chloro-3-indolyl phosphate/nitro blue (tetrazolium (BCIP/NBT) substrate (Sigma). This reaction was carried out for 5–10 min and then stopped by the addition of distilled water.

**Subcloning of the *B. henselae sucB* gene and its expression in *E. coli*.** A gene fusion of *sucB* with lacZ was used to confirm the identity of the 45 kDa antigen as SucB and allow increased expression in *E. coli*.
PCR primers from the sucB gene sequence were designed to contain a BamHI site on the 5’ end (5’-AAGGATCCCTGATGACAGGATCC-3’) and an EcoRI site on the 3’ end (5’-GGAATTC TGATCTCTACAGAATG-3’). The restriction sites allowed in-frame and directional cloning of a 1:2 kb insert containing the sucB gene into the pBluescript SK – vector (Stratagene). DNA from B. henselae was amplified through 30 cycles of 94 ℃ for 1 min, 92 ℃ for 40 s and 60 ℃ for 40 s using GeneAmp reagents, Taq polymerase and a thermal cycler (MJ Research). The resulting PCR product was digested with BamHI and EcoRI, purified and ligated to pBluescript to produce a fusion of the immunogenic protein with LacZ. The ligation was transformed into E. coli XL-1 Blue MR. Potential clones were examined by restriction endonuclease analysis and DNA sequencing to confirm the correct insert. The clone, designated pCM74, was grown to early exponential phase in LB broth, induced with 1 mM IPTG and allowed to continue to grow for an additional 2 h. Proteins were isolated as described above and resolved on a single preparative well of an SDS 4–15 % gradient PAGE minigel. Proteins were transferred to nitrocellulose, and the resulting membrane was cut into individual 3 mm strips and reacted with individual serum samples.

**Serum activity.** Individual sera positive and negative for B. henselae antibodies by IFA, sera positive for antibodies to the various other organisms listed above and sera from healthy blood donors were analysed for reactivity against the recombinant SucB protein. All sera were preadsorbed with a 1:10 dilution of a lysate of E. coli XL-1 Blue MR carrying SuperCos1, as described before, to remove non-specific antibodies reacting with proteins from E. coli.

**DNA manipulations and cloning.** Standard methods were followed for molecular biological techniques (Sambrook et al., 1989). Oligonucleotides were synthesized at the Huntsman Cancer Center peptide and DNA facility, University of Utah.

**DNA sequencing.** The DNA sequence was determined by the AB 3700 96 capillary DNA Analyser from Applied Biosystems. Synthetic oligonucleotides used as primers for DNA sequencing were synthesized by the Huntsman Cancer Center peptide and DNA facility.

**Statistical analyses.** Agreement, sensitivity and specificity of the SucB immunoblot correlation with the B. henselae IFA were calculated with 95 % confidence intervals (CI) using a two-way contingency table analysis with Yates–corrected Chi-squared test (Fleiss, 1981). A Student’s t-test was used for continuous variables.

**DNA and protein database searches.** The National Center for Biotechnology Information Services were used to consult the Swiss-PROT, GenBank and EMBL databases with the BLAST algorithm (Altschul et al., 1990; Gish & States, 1993).

**RESULTS AND DISCUSSION**

**Identification of immunoreactive clones**

Our approach for cloning the immunogenic proteins of B. henselae was similar to the method used by Anderson et al. (1995) to clone the 17 kDa antigen of B. henselae. We also used a pool of IFA-positive sera to screen a B. henselae library for immunoreactive proteins. The major difference was that we screened immunoblots of proteins separated by SDS-PAGE that were isolated from individual clones from a B. henselae cosmid library.

We chose to pool sera with titres of 1:512 or above to screen the B. henselae cosmid library so that the pooled sera would be as specific as the IFA assay can allow. This cut-off titre was based on previous studies examining the sensitivity and specificity of the IFA. Regnery et al. (1992) and Zangwill et al. (1993) increased the specificity of the B. henselae IgG IFA assay to 99 % when a titre of 1:512 was considered the cut-off. Bergmans et al. (1997) found that a cut-off titre of 1:512 was needed to obtain a specificity of 95-99 % in blood donors. Maurin et al. (2002) recommended that a titre of 1:128 should be the cut-off for the Focus Technologies IgG IFA to ensure a specificity of 95 %.

Two hundred cosmids from the B. henselae SuperCos1 library were analysed for proteins immunoreactive with the pooled sera positive by IFA for B. henselae antibodies. Numerous cosmids were identified that contained immunoreactive bands on Western blots reacting with the pooled sera. The characterization of a cosmid designated pBH96 containing a 30 kb insert and expressing a 45 kDa antigen reactive with the pooled sera is presented in this study (Fig. 1b, lane 3, white arrow). A distinct 45 kDa protein band was not seen on a Coomassie-stained SDS-PAGE gel of the E. coli strain containing cosmid pBH96 when compared to the control E. coli strain (Fig. 1a, lanes 2–3, white arrow). The antigenic 45 kDa band expressed by pBH96 on Western blot is similar in size to an approximately 45 kDa antigenic band identified in the B. henselae protein lane (Fig. 1b, lane 1).

**DNA sequence analysis of sucB gene and organization of the suc genes**

DNA sequencing of 6-5 kb of the 30 kb insert in cosmid pBH96 revealed three ORFs. Comparison of the ORFs to those in GenBank by using the BLAST algorithm showed that the ORFs encoded proteins homologous to SucA, SucB and LpdA, which are 2-oxoglutarate dehydrogenase (E1α), dihydriolpoamide succinyltransferase (E2α) and dihydriolpoamide dehydrogenase enzymes, respectively (Fig. 2). These enzymes are components of the 2-oxoglutarate dehydrogenase operon complex previously identified in E. coli and Brucella melitensis (Spencer & Guest, 1982; Zygmunt et al., 2001). The order of the sucA, sucB and lpdA genes in the operon are identical to what has been described for Bartonella vinsonii subsp. berkhoffii and similar to Brucella melitensis except that an extra ORF is located between the sucB and lpdA genes for Brucella melitensis. B. henselae sucB was determined to have 82-9 % amino acid identity to SucB of B. vinsonii subsp. berkhoffii, 85-3 % identity to SucB of Bartonella quintana and 69-8 % identity to SucB of Brucella melitensis. The sucB coding sequence consists of 1218 bp with a deduced amino acid sequence of 406 amino acids that have a calculated molecular mass of 43-6 kDa. The B. henselae sucA coding sequence consists of 2997 bp and was determined to have 76-3 % amino acid sequence identity to SucB of Brucella melitensis. The B. henselae lpdA coding sequence consists of 1404 bp and was determined to have 74-1 % amino acid sequence identity to LpdA of B. henselae.
Expression of sucB and gene fusion construction

The SucB protein has been previously recognized as an immunogenic protein of 45 kDa in Brucella-infected sheep (Zygmunt et al., 2001) and identified as an immunogenic protein of B. vinsonii subsp. berkhoufi and B. quintana by mouse polyclonal antibodies raised against heat-killed Bartonella (Gilmore et al., 2003). To confirm that SucB of B. henselae was the immunogenic protein expressed by cosmid pBH96, the coding sequence of the B. henselae sucB gene was amplified by PCR with primers designed to facilitate directional cloning into pBluescript and to produce an in-frame fusion of the antigen with a 12 kDa β-galactosidase sequence of the vector, which is inducible by IPTG. The calculated molecular mass of the fusion protein was 55.6 kDa. The induced protein was identified on Coo massie-stained SDS-PAGE (Fig. 1a, lane 5, black arrow). Both the un-induced and induced recombinant SucB immunoblotted positively with the pooled sera containing Bartonella antibodies by IFA (Fig. 1b, lanes 4 and 5).

Immunoblot reactivity of the fusion protein

Immunoblot analyses were performed to examine the reactivities of the 55.6 kDa SucB fusion protein with individual serum samples from patients with suspected CSD that were positive for B. henselae antibodies by IFA, as well as sera negative for B. henselae antibodies by IFA and sera from healthy normal control donors. Immunoblotting was performed on whole-cell proteins stained with Coomassie blue. Lanes 1, B. henselae ATCC 49793; 2, E. coli XL-1 Blue containing SuperCos1 control; 3, E. coli (pBH96); 4, un-induced E. coli (pCM74); 5, induced E. coli (pCM74) expressing the 55.6 kDa SucB fusion protein. Duplicate gel transferred to nitrocellulose and reacted with a 1:200 dilution of a human sera pool positive for B. henselae antibodies by IFA. White arrows indicate the position of the 45 kDa SucB protein. Black arrows indicate the position of the 55.6 kDa SucB fusion protein. Molecular size standards (in kDa) are shown on the left.

Fig. 1. (a) SDS-PAGE of whole-cell proteins stained with Coomassie. Lanes: 1, B. henselae ATCC 49793; 2, E. coli XL-1 Blue containing SuperCos1 control; 3, E. coli (pBH96); 4, un-induced E. coli (pCM74); 5, induced E. coli (pCM74) expressing the 55.6 kDa SucB fusion protein. (b) Duplicate gel transferred to nitrocellulose and reacted with a 1:200 dilution of a human sera pool positive for B. henselae antibodies by IFA. White arrows indicate the position of the 45 kDa SucB protein. Black arrows indicate the position of the 55.6 kDa SucB fusion protein. Molecular size standards (in kDa) are shown on the left.

Fig. 2. Organization of the B. henselae ATCC 49793 suc genes. Arrows indicate the direction of transcription.

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Nine of 50 sera negative for B. henselae antibodies by IFA (titre < 1:64) were also reactive with the SucB fusion protein for a background reactivity of 18%. Twenty-three of 100 healthy normal control sera were also reactive with the SucB fusion protein (23%). The calculated specificity of the SucB fusion immunoblot using IFA-negative sera is 82% (70.4–90.5%; CI) and using healthy normal controls is 77% (70–83.3%; CI) (Table 1).

We found that only 55 out of 100 IFA-positive serum samples reacted with the recombinant SucB protein by immunoblotting. Therefore, the sensitivity of the recombinant SucB antigen is much lower than the 92% sensitivity reported for the recombinant 17 kDa antigen of B. henselae when compared to the B. henselae IFA. The 55% sensitivity found with SucB, however, correlates with our previous study examining individual IgG Western blot patterns on IFA-positive patients (Litwin et al., 1997). We found that only 11 of 25 (44%) samples positive for B. henselae IgG antibodies by IFA showed reactivity against a 45 kDa band on Western blots of B. henselae proteins. Others have also shown that anti-Bartonella serum samples are reactive on immunoblots with proteins having molecular masses ranging from 45 to 50 kDa (Freeland et al., 1999; Liang & Raoult, 2000). In the study by Maurin et al. (1994), immunoblots showed a reactive 48.5 kDa band in B. vinsonii, B. henselae and B. quintana Western blots, when serum from a patient with HIV infection and B. quintana infection (bacillary angiomatosis) was used. This 48.5 kDa protein was also identified when mouse antisera to B. quintana or B. henselae was used to react with the Western blots.

The median IFA titre for SucB-reactive and nonreactive sera was 1:256 for both, with a range of 1:256–1:4096 for the reactive sera and 1:128–1:4096 for the non-reactive sera. The IFA titre also did not correlate with the intensity of reactivity with the recombinant SucB band on immunoblot.
For example, in Fig. 3, lanes 1 and 2 represent individual patient sera positive by IFA for *B. henselae* antibodies. Both samples had titres of 1:1024 by IFA, yet the reactivity with recombinant SucB is more intense in lane 1. There was variability of the reactivity of the individual sera with several *E. coli* proteins on the immunoblots as noted by the other bands of varying molecular mass (Fig. 3a).

**Cross-reactivity studies**

SucB reactivity was tested against sera from patients with antibodies against other bacterial species (Fig. 3b). Cross-reactivity of SucB was observed with sera positive for antibodies against *Brucella melitensis*, *M. pneumoniae*, *F. tularensis*, *Coxiella burnetii* and *R. typhi*. No cross-reactivity was observed with sera from patients with antibodies to *Chlamydia pneumoniae*, *T. pallidum*, *Ehrlichia chaffeensis*, *Q fever* (*Coxiella burnetii*), *R. rickettsii*, *Leptospira interrogans*, or *Legionella pneumophila*. A large number of IFA-negative sera (18%) and healthy normal control sera (23%) reacted with the recombinant SucB protein. This calculates to a specificity of 82 and 77% for IFA-negative and healthy control sera, respectively. This specificity, however, is actually only slightly decreased when compared to the 88% specificity observed for the 17 kDa antigen, using IFA as the gold standard. Some of this high degree of reactivity may be explained by the cross-reactivity of the SucB protein with human sera containing antibodies against *M. pneumoniae*. When a similarity search is performed on the SucB protein of *B. henselae*, it was found to be 98% identical to the corresponding region in *B. vinsonii* and *B. quintana*. Gilmore et al. (2003) screened a *B. vinsonii* subsp. *berkhoffii* library with a pool of mouse polyclonal antibodies raised against heat-killed *Bartonella* sp. cotton rat isolates. One immunoreactive clone was found to contain the 2-oxoglutarate dehydrogenase operon, sucA, sucB and lpdA. Antiserum from a mouse experimentally infected with live *Bartonella* was reactive against the recombinant *B. vinsonii* SucB protein.

Our study confirms and adds to the observations by Gilmore et al. (2003) that SucB is an important immunogenic protein for *B. henselae* infections in addition to infections with *B. vinsonii* and *B. quintana*. Gilmore et al. (2003) screened a *B. vinsonii* subsp. *berkhoffii* library with a pool of mouse polyclonal antibodies raised against heat-killed *Bartonella* sp. cotton rat isolates. One immunoreactive clone was found to contain the 2-oxoglutarate dehydrogenase operon, *sucA*, *sucB* and *lpdA*. Antiserum from a mouse experimentally infected with live *Bartonella* was reactive against the recombinant *B. vinsonii* SucB protein.

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53% similar to dihydrolipoamide acetyltransferase component (E2) of *M. pneumoniae*, an immunogenic protein in *Mycoplasma* spp. (Jan et al., 2001). Cross-reactions with *M. pneumoniae* have been described for *B. henselae* serological tests (McGill et al., 1998). Healthy people often have relatively high backgrounds of specific *M. pneumoniae* IgG antibodies in their sera, probably because of past *M. pneumoniae* infections. These antibodies remain for up to 7 years following *M. pneumoniae* infection. In a seroprevalence study by Tuuminen et al. (2000) 16% of 2- to 4-year-old children were positive for IgG antibodies to *M. pneumoniae*. Antibodies to *M. pneumoniae* continue to increase in adolescence and level off to a prevalence of about 40–50% in adulthood (Tuuminen et al., 2000).

### Cross-reactivity studies with *M. pneumoniae*

To address whether cross-reactivity with *M. pneumoniae* may cause false-positive SucB immunoblots, we tested all SucB immunoblot samples for IgG *M. pneumoniae* antibodies. We found that 40% of CSD IFA-positives, 43% of CSD IFA-negatives and 53% of healthy normal controls were positive for *M. pneumoniae* IgG antibodies. The positivity rates of the CSD IFA-positives, CSD IFA-negatives and healthy normal controls are consistent with the 40–50% prevalence observed for adult populations (Tuuminen et al., 2000).

When the *M. pneumoniae* IgG-positive samples were excluded, the number of CSD IFA-negative sera/SucB-immunoblot-negative samples decreased from ten to five and the healthy normal control/SucB-immunoblot-positive decreased from 23 to ten. Exclusion of the *M. pneumoniae* IgG-positive samples from the calculations modestly increased the specificity to 83-3 and 80-0% for IFA-negative samples and healthy normal controls, respectively. This modest increase in specificity suggests that additional cross-reactive organisms may be important in the specificity of the SucB immunoblot.

Cross-reactions of *Bartonella* serological tests have also been observed between the various *Bartonella* spp. (Dalton et al., 1995; Sander et al., 1998), and with *Coxiella burnetii* (La Scola & Raoult, 1996), *Chlamydia trachomatis* (Maurin et al., 1997), and other species, including *T. pallidum*, *R. rickettsii*, *Ehrlichia chaffeensis* and *F. tularensis* (McGill et al., 1998). Gilmore et al. (2003) found that antibodies against *Coxiella burnetii*, *F. tularensis* and *R. typhi* cross-reacted with the recombinant *B. vinsonii* SucB protein. Our study confirmed the cross-reactivity with these intracellular bacteria. Not unexpectedly, we also observed antigenic cross-reactivity of SucB with sera from patients with antibodies against *Brucella melitensis*. No cross-reactivity of *B. henselae* SucB was observed with sera from patients with antibodies to *Chlamydia pneumoniae*, *T. pallidum*, *R. rickettsii*, *Leptospira*, and *Legionella pneumophila*.

### Table 1. Calculation of sensitivity and specificity of SucB immunoblot with CSD, IFA-positive and -negative sera and normal control sera, with and without inclusion of *M. pneumoniae*-positive sera

<table>
<thead>
<tr>
<th>Test</th>
<th>M. pneumoniae-positive and -negative samples*</th>
<th>M. pneumoniae-negative samples†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSD IFA result</td>
<td>Normal controls</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>SucB +ve</td>
<td>55</td>
<td>9</td>
</tr>
<tr>
<td>SucB −ve</td>
<td>45</td>
<td>41</td>
</tr>
</tbody>
</table>

*Indirect immunofluorescence assay calculations including all samples: sensitivity 55% (49.2–59.2% CI); specificity with IFA-negatives 82% (70-4–90.5%); specificity with normal controls 77% (70–83.3%).

†Calculations using only *M. pneumoniae*-negative samples: sensitivity 49.1% (41.0–54.4% CI); specificity with IFA-negatives 82% (70.4–90.5%); specificity with normal controls 77% (70–83.3%).

Cross-reactions of *Bartonella* serological tests have also been observed between the various *Bartonella* spp. (Dalton et al., 1995; Sander et al., 1998), and with *Coxiella burnetii* (La Scola & Raoult, 1996), *Chlamydia trachomatis* (Maurin et al., 1997), and other species, including *T. pallidum*, *R. rickettsii*, *Ehrlichia chaffeensis* and *F. tularensis* (McGill et al., 1998). Gilmore et al. (2003) found that antibodies against *Coxiella burnetii*, *F. tularensis* and *R. typhi* cross-reacted with the recombinant *B. vinsonii* SucB protein. Our study confirmed the cross-reactivity with these intracellular bacteria. Not unexpectedly, we also observed antigenic cross-reactivity of SucB with sera from patients with antibodies against *Brucella melitensis*. No cross-reactivity of *B. henselae* SucB was observed with sera from patients with antibodies to *Chlamydia pneumoniae*, *T. pallidum*, *R. rickettsii*, *Leptospira*, and *Legionella pneumophila*.
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REFERENCES


