Identification and Characterization of an Immunodominant
28-Kilodalton Coxiella burnetii Outer Membrane Protein
Specific to Isolates Associated with Acute Disease

Guoquan Zhang,¹ Ho To,² Kasi E. Russell,¹ Laura R. Hendrix,¹
Tsuyoshi Yamaguchi,² Hideto Fukushi,² Katsuya Hirai,²
and James E. Samuel¹*

Department of Medical Microbiology and Immunology, Texas A & M University System Health Science
Center, College Station, Texas,¹ and Department of Veterinary Microbiology, Faculty of Agriculture,
Gifu University, Gifu, Gifu, Japan²

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Coxiella burnetii causes acute Q fever in humans and occasional chronic infections that typically manifest as
endocarditis or hepatitis. Isolates associated with acute disease were found to be distinct from a group of
chronic disease isolates by a variety of biochemical parameters and in a guinea pig fever model of acute disease,
suggesting a difference in virulence potential. We compared antigenic polypeptides among C. burnetii isolates
Immunol. 42:81-85, 1998). In order to clone the adaA gene, the N-terminal amino acid sequence of adaA was
determined and a 59-bp fragment was amplified from Nine Mile phase I DNA by PCR. The putative gene
fragment was used to screen a lambda ZAP II genomic DNA library, and an open reading frame expressing a
28-kDa immunoreactive protein was identified. Sequence analysis predicted a gene encoding an ~28-kDa mature
protein with a typical signal sequence. The adaA (acute disease antigen A) gene was detected in acute
group C. burnetii isolates but not identified in chronic group isolates by PCR and Southern blotting. A typical
signal peptide was predicted in adaA, and specific antibody to adaA reacted with the purified membrane
fraction of acute group isolates by Western blotting, suggesting that adaA is exposed on the outer surface of C.
burnetii. adaA was overexpressed in pET23a as a fusion protein in Escherichia coli to develop anti-recombinant
adaA (anti-radaA) specific antibody, which recognized a ~28-kDa band in acute group isolates but not in
chronic group isolates. In addition, immunoblotting indicates that radaA reacted with sera derived from
animals infected with acute group isolates but did not react with sera from animals infected with chronic group
isolates. These results support the idea that an adaA gene-targeted PCR assay and an radaA antigen-based
serodiagnostic test may be useful for differential diagnosis of acute and chronic Q fever.

Coxiella burnetii is an obligate intracellular bacterium that
causes acute and chronic forms of Q fever in humans. Acute Q
fever is an influenza-like illness that usually is self-limiting and
effectively treated by antibiotics (11). In contrast, chronic Q
fever is a severe, sometimes fatal disease, and patients have
responded poorly to various antibiotics (8, 20). Endocarditis is
the most common chronic manifestation, while vascular infec-
tion, bone infection, and chronic hepatitis are also reported
(21). Infection in most animals is mainly subclinical, but abor-
tion and infertility are common manifestations in ruminants
(2). Domestic animals, especially cattle, sheep, and goats, are
important reservoirs of the agent responsible for infection of
humans (7, 11).

C. burnetii has been isolated from various sources including
milk, ticks, and humans with acute and chronic Q fever world-
wide (2, 7, 8, 10). Previous studies have demonstrated that C.
burnetii isolates originating from milk, ticks, and humans with
acute Q fever differ in plasmid type (22), lipopolysaccharide
profiles (3), and chromosomal DNA restriction endonuclease
fragment patterns (5) from many isolates originating from
chronic Q fever. The differences at the phenotypic and molec-
ular levels between acute and chronic disease-associated iso-
lates suggested that there may be a virulence potential char-
acteristic of each group of isolates. Samuel et al. first reported
that C. burnetii isolates associated with acute Q fever contained
the OqP11 plasmid, while isolates associated with chronic Q
fever possessed the OqRS plasmid or the plasmid sequences
were integrated into the chromosome (22, 23). More recent
studies of several C. burnetii isolates from Europe detected
either the OqP11 plasmid-specific sequences (25, 26) or plas-
mid type OqPDV (27) in both acute and chronic disease-asso-
ciated isolates, suggesting that there was no specific gene(s) on
plasmids responsible for a specific virulence phenotype. These
data supported the notion that chronic disease could result
from isolates associated with acute disease and might result
from unique patient factors associated with immune status
(25–27). However, no chronic disease-associated organisms
have been isolated from acute Q fever patients. Therefore, it is
quite possible that there are bacterial genetic factors respon-
sible for acute disease. This hypothesis was supported in a
study by Moos and Hackstadt (17) comparing virulence of a prototype isolate from each group in guinea pigs. The acute disease group prototype isolate (Nine Mile phase I RSA493) caused infection and fever when delivered intraperitoneally with less than 10 organisms, while the chronic disease group prototype isolate (Nine Mile phase II RSA493) caused infection and fever when delivered intraperitoneally with less than 10 organisms, while the chronic disease group prototype isolate (Priscilla Q177) required at least 10^5 organisms to cause fever.

Our previous study identified a 28-kDa protein (P28) that was immunodominant in isolates originating from milk, ticks, and humans with acute Q fever but not immunogenic in isolates originating from chronic Q fever (6). This finding suggested that adaA could be associated with a pathogenic factor of acute Q fever. adaA may also have value as a marker to distinguish isolates. In order to clone and characterize the adaA-encoding gene, the N-terminal amino acid sequence of the protein was determined by protein sequencing. A 59-bp gene fragment was amplified from Nine Mile phase I DNA by PCR with one primer pair designed based on the N-terminal amino acid sequence and was used as a probe to screen a genomic library. The gene encoding P28 was cloned and sequenced. Outer membrane localization and antigenicity of adaA indicated that adaA may be a virulence factor related to acute Q fever, and the adaA gene may be a useful genetic marker for differentiation of isolates of C. burnetii.

### MATERIALS AND METHODS

**Bacterial strains, phage, and growth conditions.** Seventeen C. burnetii isolates from various clinical and geographical sources were used in this study. The original source, pathogenic characteristics, and genetic properties of these strains are summarized in Table 1. All the isolates were propagated in BGM or L929 cell cultures and purified as described elsewhere (7, 22). The bacteriophage lambda ZAP II (Stratagene, La Jolla, Calif.) was used as the vector for construction of the C. burnetii expression genomic DNA library. *Escherichia coli* XL-Blue MRF*<sup>®</sup>* (Stratagene) was cultured in Luria broth (LB) with 12.5 µg of tetracycline/ml and used as the host strain for recombinant plasmids and bacteriophage lambda ZAP II.

**Preparation of C. burnetii OMPs.** The outer membrane proteins (OMPs) of *C. burnetii* were extracted from purified *C. burnetii* Nine Mile based on the method described by Ohashi et al. (19). Briefly, purified organisms were suspended in 10 mM sodium phosphate buffer, pH 7.4, containing 1% Sarkosyl (Sigma, St. Louis, Mo.) and 50 µg each of DNase I and RNase A and incubated at 37°C for 30 min. EDTA at a final concentration of 15 mM was added to stop the nuclease reaction. The insoluble precipitates were obtained by centrifugation at 10,000 × g for 1 h, washed twice with 0.1% Sarkosyl-phosphate-buffered saline, and then resuspended in STE buffer (100 mM NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 8.0) containing 1 mM phenylmethylsulfonyl fluoride (Sigma).

**Analysis of the N-terminal amino acid sequences of adaA.** The OMPs of *C. burnetii* Nine Mile were separated by reversed discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to a polyvinylidene difluoride membrane as described elsewhere (19). The presence of adaA in the purified membrane fraction of *C. burnetii* Nine Mile was confirmed by immunoblotting as described previously (6). The portion of the polyvinylidene difluoride membrane containing adaA was excised and analyzed with the HP G1005A protein sequencing system (Takara Shuzo Co., Kyoto, Japan).

**Preparation of DNA probe specific to the P28-encoding gene.** The N-terminal amino acid sequence of adaA was determined as ENRPILNTINYQQQVEKWV by the method described by Ohashi et al. (19). Briefly, purified organisms were suspended in 10 mM sodium phosphate buffer, pH 7.4, containing 1% Sarkosyl (Sigma, St. Louis, Mo.) and 50 µg each of DNase I and RNase A and incubated at 37°C for 30 min. EDTA at a final concentration of 15 mM was added to stop the nuclease reaction. The insoluble precipitates were obtained by centrifugation at 10,000 × g for 1 h, washed twice with 0.1% Sarkosyl-phosphate-buffered saline, and then resuspended in STE buffer (100 mM NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 8.0) containing 1 mM phenylmethylsulfonyl fluoride (Sigma).

### Table 1. Original source, pathogenic characteristics, genetic group, and plasmid type of *C. burnetii* strains

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasmid type</th>
<th>Isolate</th>
<th>Phase</th>
<th>Original source</th>
<th>Disease or type</th>
<th>Passage</th>
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<tbody>
<tr>
<td>I</td>
<td>QpH1</td>
<td>Nine Mile RSA493</td>
<td>I</td>
<td>Montana, tick, 1935</td>
<td>Acute</td>
<td>307GP/1TC/1EP</td>
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<tr>
<td></td>
<td></td>
<td>Turkey RSA333</td>
<td>II</td>
<td>Turkey, human blood, 1948</td>
<td>Acute, Congolese red fever</td>
<td>31EP</td>
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<tr>
<td></td>
<td></td>
<td>Giroud RSA341 (&gt;’I)</td>
<td>I</td>
<td>Central Africa, human blood, 1949</td>
<td>Acute, Congolese red fever</td>
<td>2GP/2EP</td>
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<tr>
<td>II</td>
<td>QpRS</td>
<td>Priscilla</td>
<td>I</td>
<td>Montana, goat cotyledon, 1980</td>
<td>Abortion</td>
<td>GP/2EP</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>I</td>
<td>Panama, chiggers, 1961</td>
<td>Persistent</td>
<td>6EP</td>
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<tr>
<td></td>
<td></td>
<td>California RSA329</td>
<td>I</td>
<td>California, cow’s milk, 1947</td>
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<td>4EP</td>
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<tr>
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<td></td>
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<td>I</td>
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<td>QpDV</td>
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<td>Aortic aneurysm</td>
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<td>ME</td>
<td>FRENCH, human heart valve</td>
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*As defined by restriction enzyme banding patterns (5). b Plasmids were described elsewhere (22, 27). c Provided by Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Mont. Reference strains were determined by complement block titration (M. G. Peacock, Rocky Mountain Laboratories).

<sup>a</sup> Numbers indicate passage number; GP, guinea pig passage; TC, tissue culture; EP, egg passage; HP, hamster passage; HV, heart valve; BX, liver biopsy sample; <sup>b</sup>/ — passage prior to receipt in authors’ laboratory not known. <sup>c</sup> >—, passage history variants. <sup>d</sup> —, MAN and ME were not classified (27).
extension method with the digoxigenin DNA labeling kit (Roche Diagnostics K. K., Tokyo, Japan) and used as a DNA probe to screen the genomic DNA library of *C. burnetii* by Southern hybridization.

**Construction and screening of genomic DNA library.** A lambda ZAP II genomic DNA library was constructed as described by Macelaro et al. (9) and screened by Southern hybridization with the *adaA* gene-specific probe. Briefly, the genomic library was plated on *E. coli* XL-Blue MRF*™* to yield about 500 plaques per plate. Plates were incubated at 37°C until plaques were 1 mm in diameter. Plaques were transferred onto a nylon membrane (Amersham Pharmacia Biotech, Piscataway, N.J.) and were hybridized with the *adaA* gene-specific probe according to the protocol provided by the manufacturer (Roche Diagnostics K. K.). The positive plaques were detected by using the digoxigenin luminescent detection kit (Roche Diagnostics K. K.). In vivo excision of the pBlueScript vector along with the inserted DNA of each positive clone was performed according to the protocol of the supplier of the lambda ZAP II cloning system.

**Immunoblot analysis of *adaA* expression in *E. coli.* ** *E. coli* containing the recombinant plasmid was cultured in LB supplemented with 4 mM IPTG (isopropyl-β-D-thiogalactopyranoside) at 37°C overnight, and then cells were pelleted by centrifugation. The cell pellet was analyzed by SDS-PAGE and immunoblotting with rabbit anti-Nine Mile serum as described previously (30).

**DNA sequence analysis.** Plasmid DNAs from positive clones that expressed immunoreactive protein were isolated and purified by using the FlexyPrep kit (Amersham Pharmacia Biotech). The nucleotide sequence was partially determined by the dideoxynucleotide chain-termination method with the Thermosequenase Cy5.5 dye terminator cycle sequencing kit and SEQ4x4 personal sequencer system (Amersham Pharmacia Biotech). A BLAST search against the complete genome sequence of Nine Mile phase I (24) was achieved to identify the complete nucleotide sequence of the cloned gene. The nucleotide sequence and the deduced amino acid sequence were analyzed by the GENETYX analyzing system (Software Development Co., Ltd., Tokyo, Japan)

**Detection of the *adaA* gene from various isolates of *C. burnetii* by PCR.** A pair of primers, P28F and P28R, was designed based on the recombinant gene specific probe and used to amplify a 269-bp fragment (range from positions 369 to 637 in the open reading frame [ORF] region of the *adaA* gene) from DNAs of 17 isolates of various clinical and geographical sources. The sequences of the primers are as follows: P28F, 5′-AATAGATTCGCTCTCTCAAGCCG-3′, and P28R, 5′-TCTCGAGCGGCTGTTTTCTGAGCG-3′. PCR was performed with 2.5 U of Taq DNA polymerase (Invitrogen, Carlsbad, Calif.) in 50 μl of reaction mixture containing 20 ng of genomic DNA, 0.2 μM (each) primer, and 200 μM (each) deoxynucleotide triphosphates in 10 mM Tris-HCl (pH 8.3)–50 mM KCl–2.5 mM MgCl₂.

**RESULTS**

**Cloning the *adaA* gene.** Immunoblotting identified an immunoreactive band at 28 kDa in the purified membrane fraction of *C. burnetii* Nine Mile but did not detect reactivity in the Q217 strain (Fig. 1). The result confirmed that the 28-kDa protein corresponds to the *adaA* previously noted (6). To identify the *adaA* gene, we determined the N-terminal 31 amino acids of a 28-kDa protein from *C. burnetii* Nine Mile. Based on the amino acid sequence, we designed several primer pairs and successfully amplified a 59-bp fragment from *C. burnetii* Nine Mile DNA by PCR. The 59-bp fragment was used as a DNA probe to screen a genomic library of *C. burnetii* Nine Mile DNA. Approximately 10,000 plaques were screened by Southern hybridization with the *adaA* gene-specific probe. Forty positive plagues were purified and compared for expression of immunoreactive proteins. Coomassie brilliant blue (CBB) staining on an SDS-polyacrylamide gel identified one clone, designated p110, expressed as a protein common to all isolates tested (29, 30), was used as a control to confirm the presence of the antibodies to *C. burnetii* antigens in infection-derived sera. SDS-PAGE and immunoblotting were performed as described previously (31).

**Antiserum preparation and immunoblot analysis of *adaA* among various strains of *C. burnetii.*** The anti-*adaA* specific antibody was produced by immunization of BALB/c mice with purified *tadaA*. Briefly, BALB/c mice (6 weeks old) were immunized with purified recombinant fusion protein in adjuvant (Titermax) three times at 14-day intervals. At each immunization, mice were subcutaneously injected with 50 μl of antigen (containing 20 μg of *tadaA*) mixture with 50 μl of Titermax. After the third immunization, serum was collected and stored at −20°C.

The expression of *adaA* in various strains of *C. burnetii* was confirmed by immunoblotting with anti-*adaA* specific serum. SDS-PAGE and immunoblotting were performed as described elsewhere (31).

**Reactivity of purified *tadaA* with infection-derived sera.** The reactivity of *tadaA* with sera from guinea pigs infected with various strains of *C. burnetii* was analyzed by immunoblotting. Guinea pig serum was collected at 4 weeks post-aerosol infection with 10⁶ organisms of the Nine Mile phase I, Ohio, Q217, or Q229 strain and stored at −80°C until use. *C. burnetii* Nine Mile whole-cell lysate and purified rCom1, which is a protein common to all isolates tested (29, 30), were used as a control to confirm the presence of the antibodies to *C. burnetii* antigens in infection-derived sera. SDS-PAGE and immunoblotting were performed as described previously (31).

**Sequence analysis.** To determine the nucleotide sequence of the ORF encoding *adaA*, the purified recombinant plasmid from clone p110 was sequenced. The *adaA* gene-specific primers P28a1 and P28b1 were used in a sequence reaction to directly determine the nucleotide sequence of the *adaA* gene. The sequence of the p110 cloned insert was BLAST searched against the complete genome sequence of *C. burnetii* Nine Mile RSA493, allowing confirmation of the nucleotide sequence of...
The cloned gene (24). The nucleotide sequence (1,240 bp) from the cloned gene is shown in Fig. 2, which includes the flanking regions and the deduced amino acid sequence of P28. The adaA gene has a predicted ORF consisting of 684 bp, starting with an ATG codon at position 264 and ending with a TAG codon at position 947. The ORF is preceded by a putative ribosome-binding site, GGAGG, from 7 bp upstream of the ATG start codon. A predicted promoter sequence, TTGAA T-21 nt-TGTTAT, was found 36 bp upstream from the putative ribosome-binding site. The G+C content of the ORF coding region was 40%, which is similar to the value of C. burnetii total genomic G+C content (43%). The predicted mature P28 protein consists of 227 deduced amino acid residues and has a calculated molecular mass of 25,950 Da and a theoretical isoelectric point of 8.60. A 20-amino-acid signal peptide was also predicted for the N-terminal end of adaA. We also confirmed that the chemically determined N-terminal 31 amino acids of adaA were identical to the amino acid sequence of the mature protein deduced from the nucleotide sequence of the cloned adaA gene, which is the region immediately adjacent to a predicted signal peptide (Fig. 2). In addition, a BLAST search of GenBank with the deduced amino acid sequence of the adaA gene indicated that the ORF encoding adaA was identical to CBU0952, which was predicted by The Institute for Genomic Research gene annotation (24). These results suggest that the ORF identified in the p110 cloned gene sequence is a gene unique to C. burnetii and encodes adaA.

Detection of the adaA gene in various isolates of C. burnetii. PCR and Southern blotting were used to test and confirm...
whether the adaA gene is unique for a subgroup of isolates. The PCR result indicated that the adaA gene-specific fragment was amplified from isolates originating from humans with acute Q fever, ticks, cattle, and rodents, but PCR did not amplify any product from isolates from goats or humans with chronic Q fever (Fig. 3). Southern blotting also indicated that the adaA gene-specific probe hybridized with one band with Sall-digested DNAs of Nine Mile and Henzerling strains associated with acute Q fever but did not hybridize with any band with Sall-digested DNAs of Priscilla, Q217, Q229, MAN, and ME strains, which have been linked to chronic Q fever (data not shown). These results suggest that the adaA gene is specific for C. burnetii isolates originating from humans with acute Q fever, ticks, cattle, and rodents.

**Expression of adaA in E. coli and various isolates of C. burnetii.** The partial adaA protein of the Nine Mile strain was overexpressed as a fusion protein in pET23a. An IPTG-inducible fusion protein with a molecular mass of 28 kDa was detected in the adaA gene recombinant pET23a-transformed E. coli culture by CBB staining of the SDS-polyacrylamide gel and immunoblotting with a His-tagged specific monoclonal antibody (Fig. 4A and B, lanes 2 and 3). The expressed fusion protein was not detected in the negative control of pET23a-transformed E. coli culture (Fig. 4A and B, lanes 1). SDS-PAGE and immunoblotting also indicated that radA was successfully purified from the adaA gene recombinant pET23a-transformed E. coli culture by using ProBond resin column (Fig. 4A and B, lanes 4). To confirm that P28 is expressed by acute disease isolates but not carried by chronic disease isolates, anti-radA specific antibody was produced and used in immunoblotting with antigens of various strains of C. burnetii. Immunoblotting indicated that a ~28-kDa reaction band was detected from acute-disease-associated isolates Nine Mile and Henzerling but not observed in chronic-disease-associated isolates Priscilla and Q217 (Fig. 4C). This result confirmed that our cloned adaA gene encodes adaA and that adaA is expressed by acute-disease-associated isolates but not carried by chronic-disease-associated isolates.

**Reactivity of purified radA with sera derived from infected animals.** Figure 5 shows the immunoblots of the whole-cell antigen, rCom1, and radA with sera derived from a guinea pig infected with C. burnetii by PCR with primers P28F-P28R. Shown is an ethidium bromide-stained agarose gel electrophoretogram of PCR-amplified products. Lane 1, molecular size markers (100-bp DNA ladder); lanes 2 to 9, isolates originating from ticks, milk, and humans with acute Q fever (Nine Mile, Ohio, California, El Tayeb, Africa, Panama, Turkey, and Giround, respectively); lanes 10 to 14, isolates originating from a goat and humans with chronic Q fever (Priscilla, KQ154, KoQ229, SQ217, and GQ212, respectively); lane 15, Dugway isolate.
DISCUSSION

Cloning and characterization of adaA demonstrated that this protein is specific for acute-Q-fever-related isolates but deleted in chronic-disease-associated isolates despite geographical source, suggesting that adaA may be a virulence factor involved in the pathogenesis of acute Q fever in humans.

The predicted adaA mature protein consists of 227 amino acids and has a predicted molecular mass of 25,950 Da. This is very close to the molecular size of native adaA expressed in C. burnetii but about 2 kDa larger than the expression product of the adaA gene in E. coli (data not shown). The 25-amino-acid signal peptide is predicted in the N-terminal sequence of adaA, which is probably cleaved from the mature protein when the adaA gene is expressed in E. coli. The chemically determined N-terminal and internal peptide (data not shown) amino acid sequences of adaA were identical to the deduced amino acid sequence of the cloned adaA gene, confirming that the identified ORF encodes adaA. The cloned adaA gene recombinant pUC19 expressed adaA in E. coli DH5α cells without induction by IPTG (data not shown). A potential promoter sequence, TTGAAT-21 nt-TGTTAT, was identified in the adaA gene sequence, suggesting that the adaA gene was expressed in E. coli by using the endogenous promoter. A BLAST search of GenBank with either the nucleotide sequence or the deduced amino acid sequence for the adaA gene did not identify significant DNA or amino acid homologies, suggesting that adaA is unique to C. burnetii.

OMPs of gram-negative bacteria are employed in several important roles in the host-parasite interaction and relate to both pathogenesis and protective immunity. Due to the difficulties in cultivation and purification of C. burnetii, only a limited group of OMP-encoding genes have been characterized (4, 16, 28). Candidates for OMPs include the OpH1 plasmid-specific gene cbhe’ for a 42-kDa surface protein (15) and the OpRS plasmid-specific gene cbbe’ for a 55-kDa surface protein (12–14), which have been speculated to be virulence related and associated with acute or chronic Q fever in humans. However, recent investigations of several European isolates suggested that there were no specific genes on plasmids responsible for acute or chronic Q fever (25–27) and supported the notion that host factors may play a key role in the development of chronic Q fever. It remains unknown whether there are specific genes on the chromosome responsible for acute or chronic Q fever. Isolates from acute disease are distinct from chronic-disease-associated isolates at the molecular level (3, 5, 22) and in a guinea pig fever model of acute disease (17; K. Russell, unpublished data), suggesting different virulence potentials for groups of isolates of C. burnetii. In this study, we identified a novel ~28-kDa membrane-associated protein and demonstrated that adaA is expressed in acute group isolates but not carried by chronic group isolates, suggesting that adaA may be a virulence factor related to acute Q fever. Immunoblotting with purified adaA antigen recognized anti-adaA specific antibody from sera derived from animals infected with acute group isolates but not from sera from animals infected with chronic group isolates, suggesting that adaA is an important antigen in acute disease. Since there has been no suitable animal model developed to represent the manifestation of chronic Q fever and because there is a lack of genetic tools for C. burnetii, it is not possible to directly test whether a specific gene is related to acute or chronic disease. Recently, SCID mice have been used as a model highly sensitive to lethal challenge by an acute-disease-associated isolate of C. burnetii (1), and preliminary comparison in this model shows dramatic differences in disease from isolates which do not carry adaA (M. Andoh, unpublished data). Further studies to test whether the adaA gene can be delivered on a stable plasmid to a adaA-negative isolate may allow its role in virulence to be determined.

Since prompt antibiotic therapy could lead to a better prognosis for individual patients with chronic Q fever, developing a diagnostic method for rapid differential diagnosis of acute and chronic Q fever could be very important for control of chronic disease. Recently, based on point mutations unique to isolate groups, coml and icd genes have been used as genetic markers to distinguish acute and chronic isolates (18, 29). However, comparison of nucleotide sequences of coml and icd genes among isolates indicates that they are highly conserved between acute and chronic isolates, except for these few point mutations (18, 29). The finding that the adaA protein and the adaA gene are unique to acute group isolates can be used for development of adaA antigen-based serodiagnostic methods and/or an adaA gene-targeted PCR assay for differential diagnosis of acute and chronic Q fever in clinical samples. We have designed primers based on the nucleotide sequence of the adaA gene and used them to amplify products from DNA of various strains of C. burnetii. Amplicon products were amplified from DNA templates of isolates originating from humans with acute Q fever, ticks, cattle, and rodents but not from isolates originating from humans with chronic Q fever, suggesting that PCR for the adaA gene can be used for differentiation of acute- and chronic-disease-associated isolates. In addition, immunoblotting indicated that adaA reacted with sera derived from animals infected with acute group isolates but was not recognized by sera derived from animals infected with chronic group isolates, suggesting that an adaA antigen-based serodiagnostic test may be useful for differential diagnosis of acute and chronic Q fever in human sera. Further studies will evaluate the usefulness of an adaA gene-targeted PCR assay and an adaA antigen-based enzyme-linked immunosorbent assay for differential diagnosis of acute and chronic Q fever in clinical samples from acute and chronic Q fever patients.
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