Cloning, Sequencing, and Expression of the Gene Coding for an Antigenic 120-Kilodalton Protein of *Rickettsia conorii*

KIMBERLY W. SCHUENKE* AND DAVID H. WALKER

Department of Pathology, University of Texas Medical Branch, Galveston, Texas 77555-0609

Several high-molecular-mass (above 100 kDa) antigens are recognized by sera from humans infected with spotted fever group rickettsiae and may be important stimulators of the host immune response. Molecular cloning techniques were used to make genomic *Rickettsia conorii* (Malish 7 strain) libraries in expression vector λgt11. The 120-kDa *R. conorii* antigen was identified by monospecific antibodies to the recombinant protein expressed on construct λ4-7. The entire gene DNA sequence was obtained by using this construct and two other overlapping constructs. An open reading frame of 3,068 bp with a calculated molecular mass of approximately 112 kDa was identified. Promoters and a ribosome-binding site were identified on the basis of their DNA sequence homology to other rickettsial genes and their relative positions in the sequence. The DNA coding region shares no significant homology with other spotted fever group rickettsial antigen genes (i.e., the *R. rickettsii* 190-, 135-, and 17-kDa antigen-encoding genes). The PCR technique was used to amplify the gene from eight species of spotted fever group rickettsiae. A 75-kDa portion of the 120-kDa antigen was overexpressed in and purified from *Escherichia coli*. This polypeptide was recognized by antirickettsial antibodies and may be a useful diagnostic reagent for spotted fever group rickettsioses.

Members of the genus *Rickettsia* are obligate intracellular parasites that cannot be cultivated in cell-free media and are generally unstable when separated from host components. The zoonoses they cause are transmitted to humans by arthropods (i.e., fleas, lice, mites, and ticks), and rickettsiae spend part or all of their life cycle in the arthropod host. The proteins and antigens of the spotted fever group (SFG) rickettsiae are currently being analyzed at the molecular level through the use of DNA sequencing and PCR amplification to determine which proteins are important in immunity to rickettsial infection. However, the DNA sequences of only three protein antigen genes of the SFG rickettsiae have been published, and most of what we know about the proteins of SFG rickettsiae comes from data derived by using antisera and monoclonal antibodies (MAbs) to whole organisms.

Sera and MAbs from a variety of animals and humans infected with *Rickettsia conorii* and *R. rickettsii* show a typical recognition pattern of high-molecular-mass (between 100 and 200 kDa) immunoreactive proteins and a ladder-like lipopolysaccharide pattern (1, 7, 8, 17, 32, 34). The two major immunodominant surface proteins of *R. rickettsii*, designated rOmpA and rOmpB, have been cloned and sequenced. There is no known function of either of these proteins. The two *R. rickettsii* proteins are reported as having molecular masses of 190 and 135 kDa, respectively, representing their estimated molecular sizes when separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after heat denaturation (1, 2, 12). The molecular weight of the analogous rOmpA protein of *R. conorii* is still under investigation, as the molecular mass of this protein varies among different strains (29, 32). There has been some evidence to support the hypothesis that the heat-modifiable, surface-exposed proteins rOmpA and rOmpB stimulate protective immunity and are candidates for use in subunit vaccines (2, 19, 20, 30). However,

there has been scant evidence that purified proteins or portions of the proteins elicit a defined, characterized immune response. This information is critical in the development of possible vaccinogens against SFG rickettsial diseases.

Because rickettsiae are highly infectious and obtaining large enough amounts of purified protein antigens to produce a frequently used vaccine would be impractical, our approach was to identify a rickettsial antigen and clone its expressed gene in *Escherichia coli*. We describe the cloning and DNA sequence of the *R. conorii* 120-kDa antigen gene and demonstrate that the gene is found in a number of SFG rickettsiae. A 75-kDa portion of the antigen was expressed in *E. coli* and purified, and rabbit antibodies to this polypeptide recognize a heat-stable 120-kDa protein from five different strains of *R. conorii* and several species of rickettsiae. PCR analysis also confirmed that the gene is present in many SFG rickettsiae.

MATERIALS AND METHODS

Reagents. The λgt11 cloning system (37) was obtained through Promega Corporation (Madison, Wis.). Restriction endonucleases and media were purchased from GIBCO BRL Life Technologies, Inc. (Gaithersburg, Md.). The conversion adaptor nucleotides were the gift of Kenneth Stover, then of the Walter Reed Army Institute of Research, Washington, D.C. Synthetic oligonucleotides were custom made by Bio-Synthesis (Denton, Tex.). The Sequenase version 2.0 sequencing kit was purchased from United States Biochemical Corp. (Cleveland, Ohio).

Bacterial strains, phage, and plasmids. *R. conorii* (Malish 7 strain) was grown in Vero cells as previously described (32). Rickettsia-infected host cells were harvested with sterile glass beads, and rickettsiae were purified by discontinuous Renografin density gradient ultracentrifugation (33). *E. coli* Y1090 and Y1088 were obtained through Promega as host strains for λgt11. Phage propagation was performed as previously described (24). pUC18 and pUC19 plasmids were obtained from GIBCO BRL. *E. coli* TB1 and pMAL-c2 were obtained from New England BioLabs (Beverly, Mass.).

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* Corresponding author. Present address: Department of Biology, 0063, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0063. Phone: (619) 534-4185. Fax: (619) 534-5792.

MAbs. MAbs F2-15 and U16 were used in Western immunoblots of native *R. conorii* and *R. rickettsii* antigens. F2-15 is an *R. conorii*-specific MAb that recognizes a heat-labile 130-kDa antigen (17). U16 also recognizes an *R. conorii* heat-labile 130-kDa antigen and a heat-stable antigen that migrates at 170 to 190 kDa.

Antisera. Serum antibodies to *R. conorii* were raised in adult female New Zealand White rabbits (2- to 3-kg body weight) from D & D Rabbitry (Tyler, Tex.). Serum was shown by the microimmunofluorescent-antibody test to have a titer to *R. conorii* of greater than 1:20,000. This serum was prepared as previously described and absorbed with *E. coli* and λgt11 proteins prior to screening of the library to ensure no cross-reactivity with host cell proteins (22). Rabbit immunoglobulin G serum antibodies to a 75-kDa, high-performance liquid chromatography (HPLC)-purified portion of the recombinant *R. conorii* 120-kDa protein were also generated and purified by using conventional techniques (24).

SDS-PAGE and Western immunoblotting. Proteins were separated by SDS-PAGE with the buffers described by Laemmli (16). Gels were run at 20 mA overnight at 4°C. Proteins were transferred to nitrocellulose membranes at 200 mA and 4°C for 2 h in buffer containing 25 mM Tris, 20% methanol, and 12% glycine (28). Blots were incubated with polyclonal antisera, MAbs, or monospecific antisera and then incubated with alkaline phosphatase-conjugated immunoglobulin G and developed exactly as previously described (27).

DNA cloning. Rickettsial DNA was extracted with phenol and chloroform and then treated with DNase-free RNase. Genomic rickettsial DNA was partially cleaved with either *Sau*3A or *Hpa*II and *Hin*PI, and the resulting fragments were sized on a 0.7% agarose gel. Digested DNA was then ligated to *Eco*RI frameshift conversion adaptor nucleotides as previously described (22, 27), and the modified DNA was ligated to λgt11 arms and packaged in accordance with the manufacturer's instructions. The percentage of recombinant constructs was determined by titration of the recombinant library on lawns of *E. coli* Y1090 containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and isopropyl-β-D-thiogalactopyranoside (IPTG) (38).

The recombinant λgt11 constructs were screened for antigen production by inoculating *E. coli* Y1090 cells with the appropriate dilution of the library and immunoscreening with polyclonal antisera as previously described (38). Positive plaques were stored in SM buffer (50 mM Tris, 100 mM NaCl, 8 mM MgSO₄, 0.01% gelatin) at 4°C. Phage were plaque purified twice and immunoscreened as before to confirm positives.

Sequencing. The 6.7-kb *Eco*RI rickettsial DNA fragment from clone λ4-7 was isolated and subcloned into pUC18 or pUC19 in preparation for DNA sequencing by the chain termination method (25). DNA sequencing was accomplished with the Sequenase version 2.0 sequencing kit and [α-³²P]dATP (3,000 Ci/mmol, 10 mCi/ml; DuPont, NEN Research Products, Boston, Mass.). Double-stranded plasmid DNA (i.e., pUC18 or pUC19 containing a rickettsial DNA insert or nonrecombinant control pUC) was denatured by the alkaline method (21). Sequencing reactions and gels were prepared in accordance with the manufacturer's protocol.

DNA sequencing of some recombinant λ phage constructs was performed with a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) and *Taq* DNA polymerase in the dsDNA cycle sequencing system (GIBCO BRL) (14). Antisense primers corresponding to the very 5' end of the open reading frame (ORF) of clone λ4-7 were used to "walk up" the gene on overlapping clones. DNA sequences were analyzed with the PC/GENE sequence analysis software (IntelliGenet-

ics, Mountain View, Calif.). All sequences were confirmed by sequencing both strands.

PCR amplification of native SFG rickettsial DNAs. DNAs from *R. conorii* Malish 7 and Indian, *R. rickettsii* Sheila Smith and HLP, *R. sibirica*, *R. montana*, Thai tick typhus rickettsial strain TT118, *R. rhipicephali*, *R. akari* Kaplan, *R. australis* Cutlack, and *R. japonica* YH were isolated by lysis of infected Vero cells in PCR lysis buffer (50 mM KCl, 10 mM Tris [pH 8.3], 2.5 mM MgCl₂, 0.01% [wt/vol] gelatin, 0.45% [vol/vol] Nonidet P-40, 0.45% [vol/vol] Tween 20, 90 mg of proteinase K per ml). The mixture was kept at 56°C for 1 h and then incubated for 10 min at 95°C. The material was stored at −20°C prior to use. Control DNA lysates were prepared from uninfected Vero cells, *E. coli* Y1090, and purified λgt11 DNA in the same manner. Primers homologous to a region at the 5' end of the coding sequence were chosen and custom synthesized. One of the primers corresponds to nucleotides 483 to 503 on the sense strand and consists of the sequence 5'-AGACCCAATAACCAAGGCTGT-3'; the other primer corresponds to nucleotides 2041 to 2060 on the antisense strand and consists of the sequence 5'-ACTTGGGCTTCTACACC TTT-3' (see Fig. 2).

The DNA lysates were subjected to 30 cycles of PCR with a DNA thermal cycler and a GeneAmp PCR reagent kit (Perkin-Elmer Cetus). The cycling program consisted of 3 min at 95°C, followed by 30 cycles each of 30 s at 95°C, 1 min at 52°C, and 2 min at 72°C and a final extension cycle of 3 min at 72°C. The PCR products were subjected to agarose gel electrophoresis to visualize the predicted 1.5-kb amplified product. As a positive control to demonstrate that sufficient rickettsial DNA was contained in each DNA lysate, each lysate was also incubated with primers specific for eubacterial 16S rDNA (35) in a PCR mixture as described above. Primers P3mod and PC5, described by Wilson et al. (35), amplify a 733-bp fragment from eubacteria, which include the rickettsiae and *E. coli*.

Expression and purification of a portion of the 120-kDa antigen. The pMAL-c2 expression vector was utilized to create a maltose-binding protein (MBP) fusion protein expressed in *E. coli*. The 2,068-bp *Eco*RI-*Hin*dIII fragment of construct λ4-7 was subcloned into pMAL-c2, and *E. coli* TB1 was transformed with recombinant plasmid pMAL-c2. Cells containing the fusion plasmid were grown in accordance with the manufacturer's protocol, and expressed proteins were analyzed by SDS-PAGE and Western immunoblotting with rabbit polyclonal anti-*R. conorii* serum or rabbit anti-MBP serum as previously described. An MBP fusion of approximately 118 kDa was expressed and subsequently purified by affinity chromatography with an amylose resin column supplied with the expression and purification kit. Approximately 5 ml of fusion protein (3 mg of protein per ml) was cleaved with 30 μl of factor Xa (1 mg/ml) for approximately 24 h at 4°C. The mixture was dialyzed against 100 volumes of 10 mM Tris and 25 mM NaCl, with three changes of buffer at 2-h intervals. The cleaved proteins were concentrated through a Centriprep-30 ultrafilter (Amicon Corp., Danvers, Mass.). Final purification of the 75-kDa target protein was accomplished by separation on a reversed-phase Vydac C-4 column (25 by 0.46 cm [internal diameter]; The Sep/a/ra/tions Group, Hesperia, Calif.) with a Beckman 334 liquid chromatograph equipped with a 165 variable-wavelength UV detector. The effluent was monitored at 280 nm. The MBP and the target protein were separated over 45 min with a 55 to 46% (vol/vol) acetonitrile gradient containing 0.1% (vol/vol) trifluoroacetic acid. One-milliliter fractions were collected, dried under vacuum, and resuspended in phosphate-buffered saline. Fractions were analyzed by SDS-PAGE with discontinuous 8% polyacrylamide gels under re-
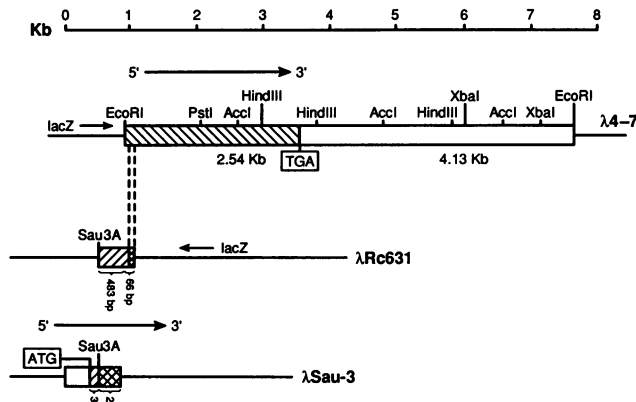
FIG. 1. λgt11 constructs for the 120-kDa antigen gene. Hatched bars represent the ORF. Also shown are several restriction endonuclease cleavage sites, a putative ATG start codon contained on construct λSau-3, and a TGA stop codon contained on construct λ4-7. The information was derived from restriction endonuclease digests of insert DNA and by sequencing the DNA inserts from each construct.

ducing conditions followed by Coomassie brilliant blue R-250 staining. All fractions that contained the protein of interest were pooled. The protein concentration was measured by using the bicinchoninic acid protein assay reagent (Pierce, Rockford, Ill.) with bovine serum albumin as the standard. The protein concentration was adjusted to 1 mg/ml, and the solution was filter sterilized through a 0.22-μm-pore-size filter and stored at −20°C.

Nucleotide sequence accession number. The primary nucleotide and deduced amino acid sequence data for the *R. conorii* 120-kDa antigen gene reported here have been deposited with GenBank (NCBI) and are available under accession number U01133.

RESULTS

Identification and analysis of recombinant constructs. Four different recombinant λgt11 libraries were created with *R. conorii* DNA, and over 10,000 recombinant phage were screened for antibody production. Approximately 25 constructs were identified with polyclonal antiserum to *R. conorii*. One construct, designated λ4-7, encodes a β-galactosidase fusion protein and migrates just above the 200-kDa molecular mass marker on Coomassie-stained, SDS-PAGE gels. MAbs to *R. conorii* Malish 7 that recognize the rOmpA and rOmpB proteins did not react with the expressed gene product produced by construct λ4-7. However, affinity purified, monospecific antibodies to the recombinant protein expressed by clone λ4-7 recognize a heat-stable *R. conorii* Malish 7 antigen that migrates at approximately 120 kDa on Western immunoblots.

Genetic analysis confirmed that construct λ4-7 contained an ORF in frame with *lacZ* and did not contain a putative ATG start codon or promoter regions. A second clone, designated λRc631, was found to have a 66-bp overlap with the 5′ end of the nucleotide sequence of the λ4-7 insert (Fig. 1). An additional 483 bp of the coding region was identified, but this was still insufficient to contain the entire coding region. A third construct, designated λSau-3, that had a 298-bp overlap with the nucleotide sequence contained on λRc631 was identified. An ORF of 3,065 bp was identified in the total of 3,896 bp sequenced; 250 bp of a noncoding region flank the 5′ end and 578 bp of a noncoding region flank the 3′ end of the ORF. The

FIG. 2. Complete nucleotide sequence and deduced amino acid sequence of the 120-kDa antigen gene of *R. conorii*. Potential −35 and −10 regions and the Shine-Dalgarno RBS are underlined. The ATG start codon at position 250 and the TGA stop codon at position 3316 are in boldface. Each corresponding amino acid is designated by the one-letter code and is shown below the triplet codon. The GenBank accession number is U01133.

DNA sequence is shown in Fig. 2. In addition, 1,896 bp were sequenced from the very 3′ end of construct λ4-7, but no potential coding regions were found in this sequence. This led us to conclude that we had correctly identified the ORF that contains the antigen gene.

The upstream region of the putative ATG start codon at position 250 was analyzed for promoter regions and the ribosome-binding site (RBS) (11, 26). The putative −35 region at nucleotides 192 to 197 (TTGACT) contains the highly conserved bases TTG found in *E. coli* −35 regions. The
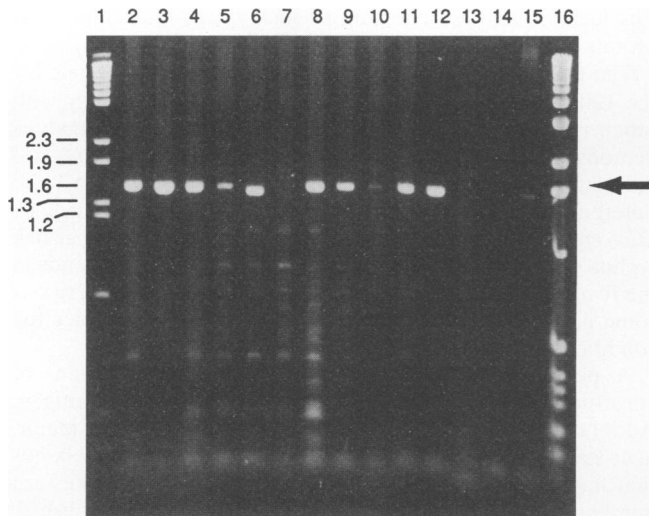
FIG. 3. PCR amplification of a portion of the 120-kDa antigen gene. The agarose gel shows PCR amplification of a 1.536-kb fragment with primers derived from our rickettsial DNA sequence and various species and strains of rickettsial genomic DNA. The arrow indicates the horizontal location of the PCR fragment. The numbers on the left are DNA sizes in kilobase pairs. Lanes: 1, λ *Bst*E DNA marker; 2, *R. conorii* Malish 7; 3, *R. conorii* Indian; 4, *R. rickettsii* Sheila Smith; 5, *R. rickettsii* HLP; 6, *R. sibirica*; 7, *R. montana*; 8, rickettsial strain TT118; 9, *R. rhipicephali*; 10, *R. akari*; 11, *R. australis*; 12, *R. japonica* YHV16; 13, Vero cell DNA lysate (negative control); 14, λ DNA (negative control); 15, *E. coli* Y1090 DNA; 16, 1-kb DNA ladder marker.
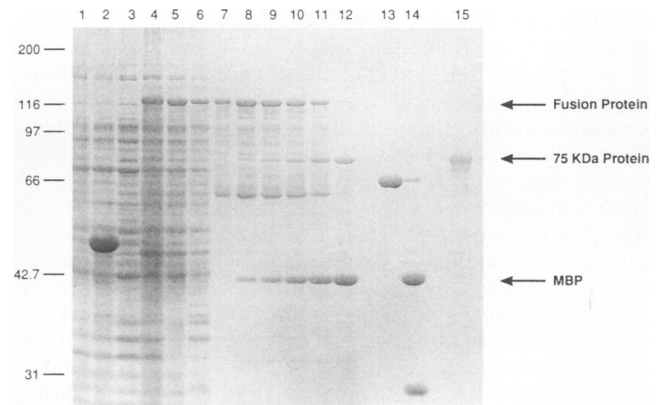

FIG. 4. Purification of MBP fusion proteins by affinity chromatography and cleavage with factor Xa. The purification process was monitored by visualizing proteins in Coomassie-stained SDS-PAGE gels. Lanes: 1, uninduced *E. coli*(pMAL-c2) vector; 2, induced *E. coli*(pMAL-c2) vector and accompanying expression of the 43-kDa MBP; 3, uninduced recombinant *E. coli*(pMAL-c2); 4, induced recombinant *E. coli*(pMAL-c2) and accompanying expression of the 118-kDa fusion polypeptide; 5, bacterial sonicate of induced recombinant *E. coli*(pMAL-c2); 6, supernatant of bacterial sonicate following centrifugation at 9,000 × g for 30 min; 7 through 12, pooled amylose column fractions following cleavage with factor Xa for 0, 1, 2, 4, 8, or 24 h, respectively; 13, MBP-paramyosin fusion protein (control); 14, MBP-paramyosin fusion protein incubated with factor Xa for 24 h (control for factor Xa); 15, HPLC-purified fraction containing the recombinant 75-kDa protein antigen. The numbers to the left are the masses of the molecular size markers in kilodaltons.

putative −10 region spans nucleotides 215 to 220 (TATGCT). The spacing between the −10 and −35 promoter sequences is 17 nucleotides, within the limit described for *E. coli* (18). The likely RBS (GAGAGGT) is indicated just upstream of the ATG start codon in Fig. 2. The sequences of the −10 and −35 promoters and RBS are similar to those of *E. coli* and to those described for other rickettsial genes (2–4, 10, 18, 26). The presumed translational initiator codon (ATG) is located at nucleotide 250 and is preceded by a termination codon in the same frame at position 235. There is an in-frame ATG at position 238, but there is no consensus RBS upstream of this codon. There is a TGA stop codon at position 3316 in the same frame as the start codon. No inverted repeats that may function as transcriptional terminators were found after the stop codon, but many other stop codons are present in the reading frame after position 3316. The coding region for the derived nucleotide sequence encodes 1,022 amino acid residues with a calculated molecular mass of 111,803 Da. The discrepancy between the calculated molecular mass and the observed recognition of a 120-kDa antigen in Western immunoblots of *R. conorii* reacted with antibodies to the recombinant protein is not clear. Posttranslational modifications such as glycosylation and phosphorylation could slow the mobility of the protein in SDS-PAGE and increase the estimated molecular mass of the protein. For consistency, we will continue to refer to the gene as the 120-kDa antigen gene.

Detection of a portion of the gene in other rickettsiae. To confirm that the homologous, overlapping sequences in constructs λ4-7, λRc631, and λSau-3 were part of the same contiguous gene and examine whether the sequence was present in other rickettsiae, two primers were used to amplify a 1.5-kb DNA fragment from various strains and species of rickettsiae by using the PCR technique. A sense strand primer at position 483 and an antisense strand primer at position 2060

were selected. The DNA templates used in the PCRs were genomic DNAs isolated from *R. conorii* Malish 7 and Indian, *R. rickettsii* Sheila Smith and HLP, *R. sibirica*, *R. montana*, *R. rhipicephali*, *R. akari*, *R. australis*, *R. japonica*, and the TT118 strain. As expected, the 1.5-kb fragment was amplified from both *R. conorii* strains (Fig. 3). In addition, all other rickettsial strain DNAs, except that of *R. montana*, showed the 1.5-kb fragment after PCR; a weak band was present in the *R. akari* lane. A weak band was also present in the *E. coli* lane, which may be the result of PCR contamination. The DNAs used in the PCR were crude lysate preparations of infected cells. To ensure that the DNA lysates from all of the strains contained sufficient rickettsial DNA for use in the PCR, eubacterial primers specific for 16S rDNA (35) were used to amplify a 733-bp fragment. As expected, the fragment was detected in all of the rickettsial strains and species and in *E. coli* Y1090 DNA (data not shown).

Overexpression of a portion of the 120-kDa antigen. Vector pMAL-c2 was used to produce a 118-kDa recombinant fusion protein with the MBP. The fusion protein was subsequently purified by affinity chromatography and cleaved with factor Xa to yield two proteins corresponding to a 43-kDa MBP and a 75-kDa antigen (Fig. 4). Subsequent purification by HPLC removed the MPB. Our yield of the recombinant 75-kDa antigen was approximately 24 mg/liter of the recombinant *E. coli*(pMAL-c2) culture. The 75-kDa rickettsial antigen reacted with convalescent-phase serum from a human infected with boutonneuse fever and antirickettsial antibodies from animals experimentally infected with *R. conorii* and *R. rickettsii* (data not shown).

Comparison of the nucleotide sequence with those of other genes. DNA sequencing data for the *R. conorii* 120-kDa antigen gene were compared with the sequences of the genes

for other rickettsial antigens (i.e., the *R. rickettsii* 17-kDa antigen gene, the rOmpA or 190/155-kDa antigen gene, and the rOmpB or 135/120-kDa antigen gene). No significant nucleotide or amino acid sequence homology was detected. Comparisons with other rickettsial genes (e.g., *R. prowazekii* citrate synthase and ATP/ADP translocase) also revealed no areas of significant homology. Both the DNA and deduced amino acid sequences of the 120-kDa antigen were compared with all of the sequences in the EMBL and GenBank nucleic acid sequence data bases, the Protein Identification Resource data bank, and the University of Geneva protein sequence data bank. No significant similarity to any other known sequence was detected. Only three antigen genes of the SFG rickettsiae have been cloned and sequenced to date. We have identified and sequenced a fourth antigen gene from *R. conorii* Malish 7 that is unique, differing from the other genes.

The antigen described in this report migrates at approximately 120 kDa. This is the same molecular mass that was originally reported for the rOmpB protein of *R. rickettsii*, but now there is general agreement (2, 12) that the actual molecular mass of rOmpB is 135 kDa. The heat-stable 120-kDa protein of *R. conorii* has been present but overlooked by our laboratory in previous antigen studies (32). By using DNA sequencing, it has been established that the gene for the 120-kDa protein reported here is not the same gene as that for rOmpB. In Western immunoblots, rabbit antibodies to a 75-kDa portion of the 120-kDa antigen recognized a distinct protein band differing from that of the 130-kDa antigen recognized by MAbs F2-15 and U-16 (data not shown).

DISCUSSION

Nucleotide sequence of the 120-kDa antigen gene and deduced amino acid sequence. The 120-kDa *R. conorii* antigen gene was identified from three overlapping constructs derived from genomic *R. conorii* DNA libraries constructed by using expression vector λgt11. DNA sequencing revealed a 3,065-nucleotide ORF that has the capacity to encode a protein of 112 kDa. The differences between the observed and calculated molecular masses may be due to posttranslational modifications, such as N-glycosylation and protein phosphorylation. A promoter region and RBS were identified and show a high degree of homology with other rickettsial genes in these regions. Codon usage in the gene resembled that of other AT-rich bacteria (36).

The deduced amino acid sequence of the *R. conorii* 120-kDa antigen was subjected to a number of computer algorithms to predict membrane-spanning regions, antigenic determinants, and secondary structure. Many α-helices were predicted according to one algorithm for globular proteins (9), but no helix that spans the membrane was predicted (6, 13, 15, 23). Lack of a putative signal peptide at the amino terminus also indicated that the protein is probably not inserted in the outer membrane (31). The hydrophobic motif (demonstrated on a hydrophobicity plot) of the *R. conorii* 120-kDa protein suggests that it is not an outer membrane protein, although there is a highly hydrophilic region at the amino terminus of the molecule. DNA sequence data reveal six potential N-glycosylation sites, but whether it is a glycoprotein has yet to be determined.

Immunoelectron microscopy of rickettsia-infected Vero cells with monospecific antiserum to the 120-kDa antigen did not show the surface-exposed locations typical of antibodies to the rickettsial outer membrane proteins (i.e., the rOmpA and rOmpB proteins) (data not shown). The colloidal gold particles marking antibody reactivity with the 120-kDa protein were located predominantly in the cytoplasm of the rickettsial cells.

The location and arrangement of such a high-molecular-mass protein within the cell cytoplasm are not known.

The nucleotide sequence and deduced protein sequence for the 120-kDa antigen gene have no significant homology with other rickettsial genes or protein sequences. PCR analysis demonstrated that the gene was present in at least two different strains of *R. conorii* (Malish 7 and Indian) and different SFG species, including *R. rickettsii*, *R. sibirica*, *R. rhipicephali*, *R. parkeri*, *R. australis*, *R. japonica*, and Thai tick typhus rickettsial strain TT118. There were two weak bands in the *R. akari* and *E. coli* lanes in Fig. 3, suggesting that there was some nonspecific cross-reactivity with these DNAs under the conditions used.

A portion of the gene, the *Eco*RI-*Hin*dIII fragment of construct λ4-7, was expressed in *E. coli* as a 75-kDa antigen. Antibodies to the purified polypeptide recognized a major, heat-stable band at 120 kDa and several weakly stained bands that migrated at approximately 100 and 112 kDa in Western immunoblots of native *R. conorii* antigens (strains Malish 7, KTT, Simko, and Indian). It is not clear whether the lower-molecular-mass bands are degradation products, represent a precursor protein, or are separate proteins with epitopes shared by the 120-kDa protein. In addition, human convalescent-phase sera from a boutonneuse fever case recognized the 75-kDa polypeptide by Western immunoblotting (data not shown). To our knowledge, this is the first report of purification of a recombinant protein from SFG rickettsiae.

The function of the rickettsial rOmpA and rOmpB antigens has not been established, although rOmpB has been characterized as an example of a structural protein termed an S-layer protein (4, 5, 12). Likewise, the 120-kDa protein in *R. conorii* described in this report has no known function. The 120-kDa protein is readily identified in SDS-PAGE gels and Western immunoblots with serum antibodies from naturally infected individuals and experimentally infected animals (7, 29, 34). The findings that the 120-kDa protein is quantitatively sufficient to be visualized in routine SDS-PAGE gels and is recognized by the humoral immune response suggest that this protein plays a role in protective immunity. Preliminary data revealing reactivity with T lymphocytes strongly support this hypothesis (data not shown). Rickettsiae are unique bacteria in that they reside in the cytosol of endothelial cells. Further investigations are required to elucidate which of the rickettsial antigens, processed by exogenous or endogenous pathways and presented in association with major histocompatibility complex molecules, stimulate the immune response. Design of an effective vaccine against SFG rickettsiae, of necessity, implies knowledge of the repertoire of antigens that stimulate T lymphocytes which secrete the key cytokine effectors of rickettsial immunity. The ideal vaccine will also stimulate proliferative expansion of cytotoxic T lymphocytes that are effectors against rickettsial antigens presented on the surface of infected endothelial cells. The identification of this protein antigen gene and the means of its purification are steps toward the goal of elucidating the interaction of rickettsiae and their vertebrate hosts.

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