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Proteomic and Immunoblot Analyses of *Bartonella quintana* Total Membrane Proteins Identify Antigens Recognized by Sera from Infected Patients[▽]

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Bartonella quintana **is a fastidious, gram-negative, rod-shaped bacterium that causes prolonged bacteremia in immunocompetent humans and severe infections in immunocompromised individuals. We sought to define the outer membrane subproteome of *B. quintana* in order to obtain insight into the biology and pathogenesis of this emerging pathogen and to identify the predominant *B. quintana* antigens targeted by the human immune system during infection. We isolated the total membrane proteins of *B. quintana* and identified 60 proteins by two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and peptide mass fingerprinting. Using the newly constructed proteome map, we then utilized two-dimensional immunoblotting with sera from 21 *B. quintana*-infected patients to identify 24 consistently recognized, immunoreactive *B. quintana* antigens that have potential relevance for pathogenesis and diagnosis. Among the outer membrane proteins, the *v*ariably expressed *o*uter *m*embrane *p*rotein adhesins (VompA and VompB), peptidyl-prolyl *cis-trans*-isomerase (PpI), and *h*emin-*b*inding *p*rotein *E* (HbpE) were recognized most frequently by sera from patients, which is consistent with surface expression of these virulence factors during human infection.**

Bartonella quintana, the agent of trench fever, is a fastidious, gram-negative, rod-shaped organism that can cause prolonged bacteremia in immunocompetent humans and severe infections in immunocompromised individuals. Humans are the only known reservoir for *B. quintana* (12), and the vector for transmission is the human body louse, *Pediculus humanus corporis* (38). *B. quintana* infections have occurred worldwide, and severe, potentially lethal complications, such as endocarditis and bacillary angiomatosis, can develop in immunocompromised patients with AIDS, cancer, and organ transplants. However, little is known about the pathogenesis of *B. quintana*, and diagnosis of human infection remains extremely challenging. To address this paucity of knowledge, we sought to identify potential membrane-associated virulence factors, as well as protective and diagnostically relevant *B. quintana* antigens, by characterizing the total membrane fraction and immunome of *B. quintana*.

Bacterial outer membrane proteins (OMP) can be important virulence factors, playing a critical role in adherence, invasion, and immune evasion during infection of the host, as well as during transmission via arthropod vectors. Many outer membrane-associated proteins that are important for pathogenesis also are consistent targets for the host immune system after infection. Workers in our lab previously identified a family of *v*ariably expressed *o*uter *m*embrane *p*roteins (Vomp) that play a role in adhesion and autoaggregation (45). To initially identify the Vomp family, we used two-dimensional (2D) sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) to visualize changes in expression of membrane proteins in sequential isolates from animals experimentally infected with *B. quintana*.

To identify additional membrane proteins of *B. quintana*, we utilized 2D SDS-PAGE to systematically characterize the total membrane protein (TMP) subproteome and to determine whether the Vomp and other identified *B. quintana* membrane proteins are recognized by sera from patients naturally infected with *B. quintana*. We constructed a protein map of the TMP of *B. quintana* by 2D gel electrophoresis and then identified individual proteins by peptide mass fingerprinting (PMF). We next performed a 2D immunoblot analysis using sera from 21 *B. quintana*-infected patients to identify the membrane-associated antigens consistently recognized by the human immune system during *B. quintana* infection. Analysis of these membrane-associated proteins provided insight into the identities of virulence factors, as well as protective and diagnostic antigens, of *B. quintana*.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and IFA testing. *B. quintana* strains were isolated from *Bartonella*-infected patients with concomitant human immunodeficiency virus infections (Table 1) and were passaged fewer than three times from frozen stocks before use. Strains were streaked onto chocolate agar plates, incubated at 37°C in candle extinction jars, and harvested for protein preparation

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TABLE 1. Reciprocal IFA titers of patient sera

Patient	*Bartonella* species isolated	Source of *B. quintana* isolate	Reciprocal IFA titer[a]	
			B. quintana	*B. henselae*
1	*B. quintana*	Skin	1,024	256
2	*B. quintana*	Skin	512	256
3	*B. quintana*	Blood	1,024	512
4	*B. quintana*	Skin	>1,024	128
5	*B. quintana*	Skin	1,024	31
6	*B. quintana*	Skin	>1,024	512
7	*B. quintana*	Skin	>1,024	>1,024
8	*B. quintana*	Abdominal mass	1,024	256
9	*B. quintana*	Skin	1,024	256
10	*B. quintana*	Skin	1,024	512
11	*B. quintana*	Skin	>1,024	>1,024
12	*B. quintana*	Skin	256	64
13	*B. quintana*	Blood	>8,192	2,048
14	*B. quintana*	Lymph node	512	128
15	*B. quintana*	Blood	512	128
16	*B. quintana*	Blood	2,048	2,048
17	*B. quintana*	Skin	>8,192	8,192
18	*B. quintana*	Blood	512	64
19	*B. quintana*	Blood	512	128
20	*B. quintana*	Skin	2,048	128
21	*B. quintana*	Blood	1,024	128
22[b]	None	NA[c]	31	31
23[b]	None	NA	31	31

[a] A reciprocal IFA titer of ≥64 indicates a positive IFA test (10, 40).
[b] Control patients 22 and 23 were both culture negative and seronegative.
[c] NA, not applicable.

after 7 days. *Bartonella* antibody titers were determined for each patient serum sample by indirect immunofluorescent antibody (IFA) testing. The IFA test for *Bartonella* antibodies was developed at the CDC (10, 40). Patient serum was diluted twofold to 1:1,024, and a reciprocal titer to *Bartonella henselae* or *B. quintana* of ≥64 was considered a positive result based on previous studies (10, 40). Although the antigenic profile of *B. quintana* grown with Vero cells for IFA analysis and the antigenic profile of bacteria grown on agar for immunoblotting may differ somewhat, culture on agar was necessary to generate a sufficient mass of bacteria and to maintain bacterial cell fractions that did not contain eukaryotic cells.

Protein preparation. Subcellular fractions were obtained by using methods described previously (32, 42). Bacteria were harvested from chocolate agar plates, washed twice with phosphate-buffered saline (PBS) (pH 7.4), and pelleted by centrifugation with a tabletop microcentrifuge for 2 to 3 min at 4°C at the maximum speed. The final pellet was resuspended in 10 mM HEPES buffer. We added a protease inhibitor cocktail [20 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride (Calbiochem, San Diego, CA); 1 mg/ml leupeptin, 0.36 mg/ml E-64, and 5.6 mg/ml benzamidine, all obtained from Sigma, St. Louis, MO; 50 mM EDTA] and incubated the preparation on ice for 10 min. The bacterial cells were disrupted with five 1-min bursts using a sonicator (Labsonic U sonicator; B. Braun Biotech, Inc., Allentown, PA) with cooling on ice between bursts. A small aliquot was removed and saved for whole-cell lysate preparation. Cellular debris was pelleted by centrifugation at 4,300 × *g* for 30 min at 4°C with a Sorvall centrifuge (SS-34 rotor; Thermo Electron, Asheville, NC). The supernatant was transferred to Ultra-Clear ultracentrifuge tubes (13 by 51 mm; Beckman, Palo Alto, CA) and centrifuged at 100,000 × *g* with an L8-M ultracentrifuge for 1.5 h at 4°C using an SW55Ti rotor (Beckman). The supernatant was removed and saved for cytosolic protein preparation. The pellet was resuspended either in 10 mM HEPES for TMP preparation or in 1% (wt/vol) *N*-lauryl sarcosine (Sigma) in 10 mM HEPES to separate the OMP. The lauryl sarcosine suspension was incubated at room temperature for 30 min and then pelleted by ultracentrifugation at 100,000 × *g* for 1.5 h at 4°C. The supernatant containing the inner membrane proteins (IMP) was saved, and the OMP pellet was resuspended in 10 mM HEPES and treated with nuclease (50 mM $MgCl_2$, 100 mM Tris [pH 7.0], 500 μg/ml RNase, 1 mg/ml DNase [Sigma]). OMP were then washed twice in 10 mM HEPES and pelleted by centrifugation at 40,000 × *g* for 30 min at 4°C with a Sorvall centrifuge (SS-34 rotor). The final TMP preparation was treated with nuclease and pelleted in the same way. The cytosolic preparation was precipitated with 45% ammonium sulfate (Sigma) in 0.01 M Tris (pH 7.0) and incubated on ice for 45 min. The precipitated proteins were pelleted by centrifugation at 19,000 × *g* for 30 min at 4°C. The resulting pellet was resuspended in cold PBS and dialyzed overnight against PBS using a D-tube dialyzer maxi (molecular weight cutoff, 3,500 Da; Novagen, Darmstadt, Germany). The dialyzed proteins were concentrated using an Amicon Ultra-4 centrifuge filter (Millipore, Bedford, MA). The IMP preparation was concentrated in the same way. Protein concentrations were determined using a MicroBCA protein assay (Pierce, Rockford, IL), and proteins were separated by one-dimensional (1D) SDS-PAGE to confirm that subcellular fractions were separated. All fractions were frozen at −80°C until they were used.

2D gel electrophoresis and transblotting. 2D gel electrophoresis was performed using the method of O'Farrell (35) by Kendrick Labs, Inc. (Madison, WI), as follows. Protein pellets were dissolved in 200 ml of SDS boiling buffer (5% SDS, 10% glycerol, 60 mM Tris [pH 6.8]) without β-mercaptoethanol, and protein concentrations were determined using a bicinchoninic acid assay (Pierce). Protein samples were then diluted to obtain a concentration of 2.0 or 4.0 mg/ml in SDS boiling buffer containing 5% β-mercaptoethanol and boiled for 5 min. Isoelectric focusing was carried out in glass tubes with an inside diameter of 2.0 mm using 2% pH 4 to 8 ampholines (BDH; obtained from Hoefer Scientific Instruments, San Francisco, CA) for 9,600 V · h. For the TMP preparations 100 μg of protein was loaded, and for the OMP preparations 200 μg was loaded. After equilibration for 10 min in buffer O (10% glycerol, 50 mM dithiothreitol, 2.3% SDS, 0.0625 M Tris [pH 6.8]), the tube gels were laid on top of 10% acrylamide slab gels (thickness, 0.75 mm), and SDS slab gel electrophoresis was carried out for about 4 h at 12.5 mA/gel. The gels were stained with either Coomassie brilliant blue R-250 or silver stain (34). The Coomassie brilliant blue-stained gels were maintained wet in 10% acetic acid between sheets of filter paper until spot excision and subsequent mass spectrometry analysis; the silver-stained gels were dried between sheets of cellophane. After slab gel electrophoresis, a duplicate gel was transblotted onto a polyvinylidene difluoride membrane (Immobilon-P; Millipore).

Immunoblotting of *B. quintana* membrane proteins with human sera. To identify immunoreactive proteins in the TMP fraction of *B. quintana*, proteins separated by 2D SDS-PAGE were transferred to polyvinylidene difluoride membranes, which were then blocked overnight at 4°C with 5% milk in TBST (150 mM NaCl, 10 mM Tris-HCl [pH 8.0], 0.5% Tween 20, 0.2% sodium azide). The membranes then were washed three times (5 min each time) in TBST containing 0.5% bovine serum albumin (Sigma) and once in TBST. Patient sera (primary antibody) were inactivated with 0.5% Nonidet P-40 (Roche, Mannheim, Germany) and diluted 1:50 in TBST. Each membrane was placed in a heat-sealable bag containing the primary antibody and vigorously shaken for 2 h at room temperature. The membranes were washed, blocked for 30 min in 1% milk in TBST, and then washed again. Secondary antibody (alkaline phosphatase-conjugated goat anti-human immunoglobulin G; Zymed Laboratories, Inc., South San Francisco, CA) was diluted 1:5,000 and incubated with the membranes for 30 min at room temperature. The membranes were washed and developed using alkaline phosphatase buffer (100 mM Tris-HCl [pH 9.5], 100 mM NaCl, 5 mM $MgCl_2$), nitroblue tetrazolium, and 5-bromo-4-chloro-3-indolylphosphate (BCIP) (Promega, Madison, WI).

To corroborate the immunoreactivity of *B. quintana* proteins identified by the immunoblotting described above, a λ genomic expression library of *B. quintana* strain JK31 was screened using serum from a *Bartonella*-infected patient. The library was generated with a Sau3AI partial digest of *B. quintana* chromosomal DNA and the lambda-ZAP Express vector used according to the manufacturer's instructions (Stratagene, La Jolla, CA) and was screened by lifting plaques onto isopropyl-β-D-thiogalactopyranoside (IPTG)-impregnated nitrocellulose (27), followed by immunoblotting, as previously described (30). The initial screening was performed for 3 h at 25°C using polyclonal rabbit anti-*B. quintana* antiserum (1:1,000 dilution of serum generated by intravenous immunization with *B. quintana*, as described previously [43], except that the formalin treatment was omitted). Human antibody recognition of positive plaques was verified by using serum (1:50 dilution) from a patient with a *Bartonella* infection; plaque lifts were probed for 16 h at 25°C. Plaques identified as positive for both human and rabbit antisera were isolated, replaqued, and rescreened to ensure clonality. Phagemid contents were excised and rescued with *Escherichia coli* XLOLR (Stratagene), and then plasmids were purified and sequenced (29). Data were analyzed using the Chromas (Technelysium), MacVector (Accelrys, San Diego, CA), and BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) software. The protein profiles for each strain were analyzed to identify seroreactive protein species by SDS-PAGE and immunoblotting, as previously described (27). Molecular mass values for reactive proteins were cross-referenced to predicted mass values for plasmid-encoded proteins determined by sequence analysis.

In-gel trypsin digestion. Individual protein spots were excised from the Coomassie brilliant blue-stained 2D gels and cut into 1-mm^2 pieces. The stain was removed from the gel pieces by incubating the pieces with 25 mM ammonium bicarbonate (NH_4HCO_3) (Sigma) in 50% acetonitrile (Sigma) overnight with gentle vortexing. After removal of the supernatant, gel pieces were dried with a SpeedVac and then reduced with 10 mM dithiothreitol (Sigma) for 1 h at 56°C, followed by alkylation with 55 mM iodoacetamide (Sigma) for 45 min at room temperature in the dark. Next, the gel pieces were washed with 25 mM NH_4HCO_3, dehydrated with 100% acetonitrile and dried with a SpeedVac. Porcine trypsin (12.5 ng/μl in 25 mM NH_4HCO_3; Promega) was added, and the gel pieces were allowed to rehydrate for 1 h on ice. The excess trypsin solution was removed, and 25 mM NH_4HCO_3 was added to cover the gel pieces. The preparations were digested at 37°C overnight. Next, the supernatant (postdigestion) was transferred to a clean Eppendorf tube, and tryptic peptides were extracted from the gel pieces by vortexing them for 15 min with 50% acetonitrile–50% H_2O–0.1% trifluoroacetic acid (Pierce). The peptide extract was combined with the postdigestion supernatant, and the total volume was reduced to approximately 10 μl with a SpeedVac. The concentrated peptide extracts were desalted using C_{18} ZipTips (Millipore) and were eluted with 3 to 5 μl of 50% acetonitrile–0.1% trifluoroacetic acid. The peptides were stored at −80°C until they were used.

Mass spectrometry. PMF was used for protein identification. Peptide extracts were mixed with a matrix solution containing α-cyano-4-hydroxycinnamic acid (2 mg/ml in 50% acetonitrile–0.1% trifluoroacetic acid) at a 1:1 (vol/vol) ratio directly on a stainless steel target. A matrix-assisted laser desorption ionization—time of flight mass spectrometry analysis was performed in the reflector, positive-ion mode in the mass-to-charge ratio (*m*/*z*) range from *m*/*z* 800 to *m*/*z* 4000 utilizing a Voyager DE STR matrix-assisted laser desorption ionization—time of flight mass spectrometer (Applied Biosystems, Foster City, CA). Each mass spectrum was calibrated internally using trypsin autolysis product masses. Mass spectra were processed (baseline adjustment, noise filtering, and de-isotoping) to produce a list of monoisotopic, monoprotonated molecular ion masses. Monoisotopic peak lists were submitted to the Mascot Peptide Mass Fingerprint (http://www.matrixscience.com) search engine for analysis. Searches that interrogated the *Eubacteria* protein database within the MSDB (ftp://ftp.ncbi.nih.gov/repository/MSDB/msdb.nam) sequence database were performed. Validation of the results was based on the top hit score, a requirement for high precision of mass measurement (defined as a low standard deviation, <25 ppm, of mass assignment errors for all matching peptide masses detected within a sample spot) and a minimum of 30% sequence coverage.

Selected protein identities were confirmed by high-performance liquid chromatography-tandem mass spectrometry. Liquid chromatographic separation was performed with an Ultimate capillary high-performance liquid chromatography system (Dionex/LC Packings, Sunnyvale, CA) equipped with a PepMap trap column (Dionex/LC Packings) and a reversed-phase C_{18} nanocolumn (packed in house; inside diameter,75 μm; length, 15 cm; pore size, 100 Å; particle size, 3 μm) and a Famos Micro autosampler. A 3- to 4-μl aliquot of peptide extract was loaded onto the trap column with loading solvent (0.1% formic acid) at a flow rate of 20 μl/min. The trap column was washed with the loading solvent for 3 min before it was switched in line with the reversed-phase nanocolumn. The nanocolumn mobile phase flow rate was 325 nl/min, and the nanocolumn was maintained at the ambient temperature. The nanocolumn was equilibrated with 2% solvent B (80% acetonitrile, 20% H_2O, 0.08% formic acid) and 98% solvent A (2% acetonitrile, 98% H_2O, 0.1% formic acid) for 20 min prior to sample injection. Peptides were separated using a binary gradient that consisted of a 5-min isocratic wash with 2% solvent B, followed by a linear gradient from 2% solvent B to 50% solvent B over 45 min and then by a column cleanup step consisting of 95% solvent B for 7 min. The column effluent flowed directly into a nanoelectrospray ion source (Protana, Odense, Denmark) in a QSTAR XL quadrupole/quadrupole time of flight mass spectrometer (Applied Biosystems). Proteins were identified by isolating sequentially eluting peptide populations with a single *m*/*z* value in the mass spectrometer, fragmenting each population, and determining the masses of the peptide fragment ions. The experimentally determined peptide fragment ion masses were matched, within a window of ±0.2 Da, to theoretical fragment ion masses generated by in silico fragmentation of all theoretical tryptic peptides derived from *Eubacteria* protein sequences in the MSDB database.

In silico analysis of proteins that were identified. In addition to spot identification by PMF, we used PSORTb v.2.0 to predict protein localization based on signal peptides, transmembrane helices, homology to proteins whose localization is known, and amino acid composition and motifs (14; http://www.psort.org/psortb/). Identities of protein families were determined using Pfam (4, 11; http://www.sanger.ac.uk/Software/Pfam/). Grand average of hydropathy was used to evaluate the hydrophilicity and hydrophobicity of each protein along its amino acid sequence (23; http://us.expasy.org/tools/protparam.html).

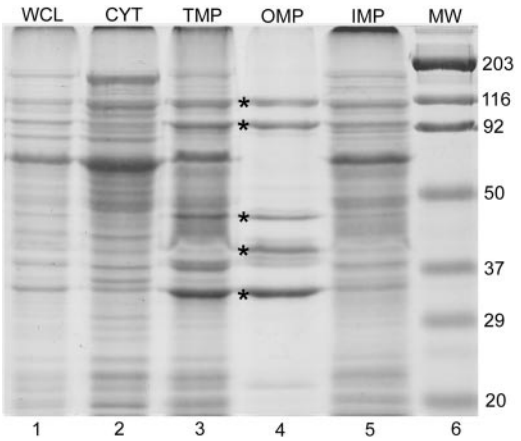

FIG. 1. Subcellular fractions of proteins from *B. quintana* wild-type strain JK31 visualized on a Coomassie blue-stained 1D SDS-PAGE gel. Proteins were fractionated using a lauryl sarcosine method, separated on a 10% acrylamide gel, and stained with Coomassie blue. The following subcellular fractions were loaded in individual lanes: whole-cell lysate (WCL), cytoplasmic fraction (CYT), TMP, sarcosine-insoluble OMP, and sarcosine-soluble IMP. Distinct protein profiles of the fractions can be distinguished, and the prominent bands in the OMP preparation are indicated by asterisks. The positions of molecular weight markers (lane MW) are indicated on the right.

Evaluation of gels and immunoblots. Spot detection for gels and blots was performed using the 2D Evolution software (Nonlinear Dynamics, Durham, NC). Spot detection for the master gel was performed manually due to nonspecific spot detection. A silver-stained gel of *B. quintana* JK31 proteins was used as the master protein profile, and each spot was assigned a number. Immunoreactive spots identified on the blots were automatically matched with the master gel, and additional spots detected visually were added manually. To allow comparisons across immunoblots, the background was subtracted using a fully automated method with the Evolution software, called Lowest on Boundary, and was determined by tracing a line just outside the boundary of each spot and then using the lowest pixel intensity that was encountered during this process as the background intensity for that spot. Additionally, each blot was normalized to a single common spot in the blot to eliminate differences in spot intensity due to immunoblot development. The volume of each spot was then calculated by dividing the pixel intensity by the area of the spot. Means, medians, and ranges of volumes were determined.

RESULTS

Enrichment and separation of *B. quintana* membrane proteins resulted in identification of distinct membrane fractions by 1D and 2D SDS-PAGE. OMP were isolated from *B. quintana* JK31 using lauryl sarcosine fractionation. This method has been used to enrich OMP from a number of bacterial species (3, 31, 39) and was also utilized to separate the OMP from the IMP of *B. henselae* (42). We initially compared the protein profiles of the subcellular fractions of *B. quintana* after separation by 1D SDS-PAGE, followed by Coomassie blue staining (Fig. 1). Enrichment of the OMP and IMP from the TMP preparation was evident when the proteins were compared with proteins from the cytosolic preparation. There were prominent bands in the OMP preparation at approximately 116, 93, 45, 40, and 34 kDa (Fig. 1). These prominent OMP bands either were observed exclusively with the OMP fraction or were highly enriched in the OMP fraction compared with the IMP fraction. Coomassie blue staining of proteins sepa-

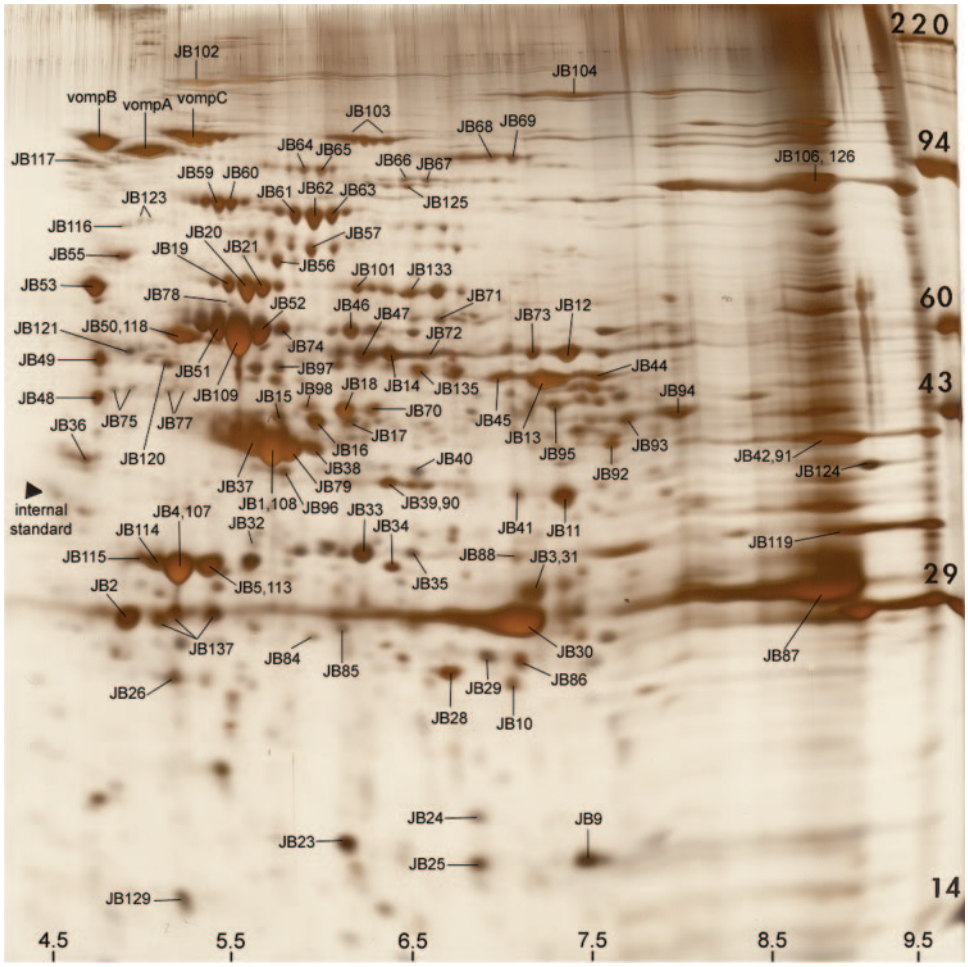

FIG. 2. 2D map of the total membrane subproteome of *B. quintana* wild-type strain JK31. Membrane proteins were separated by isoelectric point in the first dimension and then by molecular mass in the second dimension. Proteins were visualized by silver staining, and spots were excised individually and then identified by PMF. Nearly 300 protein spots were visualized, and 110 proteins, representing 60 unique *B. quintana* proteins, were identified by PMF. Each JB number indicates a protein for which a PMF identity was obtained; these numbers correspond to the protein identities shown in Table 2, Table 3, and Table 4. The arrowhead on the left indicates an internal standard, tropomyosin, which was included with each sample. This standard migrated as a doublet with a molecular mass of 33 kDa and a pI of 5.2 (for the lower spot). The positions of molecular weight markers are indicated on the right, and pI values are indicated at the bottom.

rated on a gel containing a lower percentage of acrylamide revealed that the 116-kDa bands in the OMP and IMP fractions were actually at slightly different molecular masses (data not shown).

Mass spectrometry analysis identified distinct proteins that comprise the TMP subproteome of *B. quintana*. The TMP and OMP preparations were resolved by 2D SDS-PAGE, followed by silver staining. The resolution of individual spots from gel to gel and for different protein preparations was highly reproducible. We visualized more than 300 distinct protein spots in the TMP preparation in a pI range from 4.5 to 9.5 and in a molecular mass range from 14 to 220 kDa (Fig. 2). Protein spots were numbered, excised from Coomassie blue-stained gels, subjected to in-gel tryptic digestion, and submitted for protein identification by PMF. We excised 137 spots and identified 110 separate protein spots (Table 2). Some spots were not positively identified because of the low concentration of protein and/or contamination with human keratin. The 110 spots identified by PMF correspond to 60 *B. quintana* genes. With the exception of a few spots that stained intensely with silver stain but not with Coomassie blue, most protein spots that were visualized were identified.

A number of the *B. quintana* membrane proteins identified appeared as protein isoforms or families. The protein product of a single gene can appear as several protein spots on a 2D gel due to posttranslational modifications; these isoforms are usually visualized as a horizontal pattern of spots at the same molecular weight. The modifications of bacterial proteins can include phosphorylation, glycosylation, methylation, deamidation, and biotinylation, each of which can affect the charge and the isoelectric point. Of the 60 unique proteins that we identified, 18 had at least two isoforms, and these proteins included proteins that play a role in energy metabolism (AcnA, AtpA, AtpD, PpdK, SucB, and SucD), protein fate (ClpB and MopA), protein synthesis (FusA, RpsA, and Tuf1), transcription (Pnp and Rho), purine ribonucleotide synthesis (GuaB and GlyA), and virulence (HbpE, HbpD, VompA, VompB, and VompC).

TABLE 2. *B. quintana* proteins identified in this study by PMF using matrix-assisted laser desorption ionization–time of flight mass spectrometry

Spot	Gene	Locus	UniProt accession no.	TIGR protein description	Predicted pI	Predicted mol wt (10^3)	% Mass values matched	Sequence coverage (%)	Pfam model	Pfam E-value[a]	PSORTb localization	PSORTb probability[b]	GRAVY value[c]
JB1	*tuf1*	BQ08250	Q6FZC0	Elongation factor tu	5.1	42.9	42	36	GTP binding	1.40E-98	Cytoplasmic	9.97	−0.153
JB2	*hbpD*	BQ04010	Q8KP12	Hemin-binding protein D	9.0	32.7	30	21	Porin	2.00E-14	Outer membrane	10.00	−0.247
JB3	*hbpD*	BQ04010	Q8KP12	Hemin-binding protein D	9.0	32.7	24	19	Porin	2.00E-14	Outer membrane	10.00	−0.247
JB4	*hbpE*	BQ08430	Q8KP11	Hemin-binding protein E	4.9	33.0	17	37	Porin	0.014	Outer membrane	9.93	−0.190
JB5	*hbpE*	BQ08430	Q8KP11	Hemin-binding protein E	4.9	33.0	24	31	Porin	0.014	Outer membrane	9.93	−0.190
JB9	NA[d]	BQ10800	Q6FYU6	Hypothetical protein	6.5	19.8	41	56	Reductase/antioxidant	5.10E-23	Unknown		−0.428
JB10	*rplY*	BQ03290	Q6G0F	50S ribosomal protein L25	6.1	22.8	50	36	RNA-binding protein	1.10E-20	Unknown		−0.127
JB11	*sucD*	BQ13430	Q6FYD2	Succinyl-coenzyme A synthetase alpha chain	6.5	31.1	37	35	Ligase	2.60E-55	Unknown		0.060
JB12	*guaB*	BQ01690	Q6G181	Inosine-5′-monophosphate dehydrogenase	6.6	52.9	54	45	Dehydrogenase/ reductase	1.20E-224	Cytoplasmic	8.96	−0.020
JB13	*atpA*	BQ12250	Q6FYM1	ATP synthase alpha chain	6.4	55.5	58	44	ATP synthase	6.40E-123	Unknown		−0.061
JB14	*atpD*	BQ12230	Q6FYM3	ATP synthase beta chain	5.6	57.1	52	33	ATP synthase	2.30E-98	Cytoplasmic	8.96	−0.128
JB15[e]	*sucB*	BQ13410	Q6FYD4	Dihydrolipoamide succinyltransferase	5.2	43.8	29	18	Acyltransferase	6.70E-138	Cytoplasmic	8.47	−0.027
JB16[e]	*sucB*	BQ13410	Q6FYD4	Dihydrolipoamide succinyltransferase	5.2	43.8			Acyltransferase	6.70E-138	Cytoplasmic	8.47	−0.027
JB17[e]	*sucB*	BQ13410	Q6FYD4	Dihydrolipoamide succinyltransferase	5.2	43.8	38	18	Acyltransferase	6.70E-138	Cytoplasmic	8.47	−0.027
JB18	*rho*	BQ13580	Q6FYB7	Transcription terminator factor rho	5.3	47.3	45	66	ATP synthase	4.10E-80	Cytoplasmic	8.96	−0.212
JB19	*rpsA*	BQ00860	Q6G0X5	30S ribosomal protein s1	5.0	62.8	54	43	RNA binding	1.50E-29	Cytoplasmic	8.96	−0.343
JB20	*rpsA*	BQ00860	Q6G0X5	30S ribosomal protein s1	5.0	62.8	63	55	RNA binding	1.50E-29	Cytoplasmic	8.96	−0.343
JB21	*rpsA*	BQ00860	Q6G0X5	30S ribosomal protein s1	5.0	62.8	58	51	RNA binding	1.50E-29	Cytoplasmic	8.96	−0.343
JB23	*nusG*	BQ07180	Q6FZL4	Transcription antitermination protein	5.2	20.1	18	43	Transcription factor	2.00E-50	Cytoplasmic	8.96	−0.319
JB24	*ssb*	BQ07860	Q6FZF9	Single-strand binding protein	5.8	19.4	17	89	Single strand binding	2.80E-29	Unknown		−0.899
JB25	*ibpA2*	BQ05230	Q6G023	Small heat shock protein	6.0	19.3	26	48	Crystallin	4.10E-19	Cytoplasmic	8.96	−0.604
JB26	*nuoC*	BQ05660	Q6FZY9	NADH dehydrogenase I, C subunit	4.9	23.4	8	47	Dehydrogenase	9.10E-46	Cytoplasmic	8.96	−0.401
JB28	*ctrA*	BQ09460	Q6FZ43	Cell cycle transcriptional regulator	6.0	26.3	24	85	Response regulator receiver	4.70E-31	Cytoplasmic	9.97	−0.130
JB29	*purC*	BQ07470	Q6FZJ2	Phosphoribosylaminolmidazole-succinocarboxamide synthase	6.3	29.1	27	61	Synthetase	2.20E-88	Cytoplasmic	8.96	−0.324
JB30	*hbpD*	BQ04010	Q8KP12	Hemin-binding protein D	9.0	32.7	18	40	Porin	2.00E-14	Outer membrane	10.00	−0.247
JB31	*hbpD*	BQ04010	Q8KP12	Hemin-binding protein D	9.0	32.7	17	35	Porin	2.00E-14	Outer membrane	10.00	−0.247
	prsA	BQ03310	Q6G0F7	Ribose-phosphate pyrophosphokinase	6.6	33.6	16	47					
JB32	*tsf*	BQ07000	Q9XCM5	Elongation factor ts	5.0	32.5	19	66	Elongation factor TS	3.30E-86	Cytoplasmic	9.26	−0.047
JB33	*ppi*	BQ01880	Q6G0Q7	Peptidyl-prolyl *cis-trans*-isomerase	5.6	35.7	36	68	Rotamase	1.20E-38	Outer membrane	9.92	−0.415
JB34	*etfA*	BQ09680	Q6FZ25	Electron transfer flavoprotein alpha subunit	5.7	32.5	21	34	Flavin adenine dinucleotide binding	1.80E-54	Unknown		0.157
JB35	*ppi*	BQ01880	Q6G0Q7	Peptidyl-prolyl *cis-trans*-isomerase	5.6	35.7	38	57	Rotamase	1.20E-38	Outer membrane	9.92	−0.415
JB36	*rpoA*	BQ07990	Q6FZE6	DNA-directed RNA polymerase alpha chain	4.4	37.7	13	74	Polymerase	9.40E-54	Cytoplasmic	9.97	−0.221
JB37	*tuf1*	BQ08250	Q6FZC0	Elongation factor tu	5.1	42.9	26	63	GTP binding	1.40E-98	Cytoplasmic	9.97	−0.153
JB38	*tuf1*	BQ08250	Q6FZC0	Elongation factor tu	5.1	42.9	18	66	GTP binding	1.40E-98	Cytoplasmic	9.97	−0.153
JB39	*recA*	BQ07950	Q6FZF0	Recombinase A protein	5.3	37.5	23	79	DNA recombination	2.10E-246	Cytoplasmic	8.96	−0.052
JB40	*hrcA*	BQ00490	Q6G1E5	Heat-inducible transcription repressor	5.9	39.7	26	62	HrcA C-terminal domain	5.80E-76	Cytoplasmic	8.96	−0.253
JB41	*sucD*	BQ13430	Q6FYD2	Succinyl-conenzyme A synthetase alpha chain	6.5	31.1	12	39	Ligase	2.60E-55	Unknown		0.060
JB42	*omp43*	BQ09890	Q6FZ11	Outer membrane protein	9.5	44.0	27	50	Porin	1.80E-51	Outer membrane	9.93	−0.291
JB44	*atpA*	BQ12250	Q6FYM1	ATP synthase alpha chain	6.4	55.5	34	54	Synthase	6.40E-123	Unknown		−0.061
JB45	*atpA*	BQ12250	Q6FYM1	ATP synthase alpha chain	6.4	55.5	30	53	Synthase	6.40E-123	Unknown		−0.061

JB46	*ntrX*	BQ05010	Q6G180	Nitrogen regulation protein	5.2	50.3	18	50	Sigma-54 interaction	4.00E-97	Cytoplasmic	9.97	−0.189
JB47	*atpD*	BQ12230	Q6FYM3	ATP synthase beta chain	5.6	57.1	23	42	ATP synthase	2.30E-98	Cytoplasmic	8.96	−0.128
JB48	*ftsZ*	BQ08800	O69075	Cell division protein FtsZ homolog	5.1	63.8	31	39	GTPase	5.40E-95	Cytoplasmic	9.12	−0.293
JB49	*pdhB*	BQ04920	Q6G169	Pyruvate dehydrogenase E1 component beta subunit	4.5	49.3	28	63	Transketolase	1.10E-77	Cytoplasmic	8.96	−0.006
JB50	*tig*	BQ07290	Q6F2K6	Trigger factor	4.7	53.5	34	53	Trigger factor protein	1.40E-52	Cytoplasmic	8.96	−0.565
JB51	*mopA*	BQ10750	033964	Chaperonin protein GroEL	4.9	57.6	26	53	Chaperonin	1.10E-194	Cytoplasmic	9.97	−0.041
JB52	*mopA*	BQ10750	033964	Chaperonin protein GroEL	4.9	57.6	44	76	Chaperonin	1.10E-194	Cytoplasmic	9.97	−0.041
JB53	*nusA*	BQ02050	Q6G0P0	N utilization substance protein A	4.5	60.7	34	67	RNA binding	8.60E-07	Cytoplasmic	8.96	−0.333
JB55	*dnaK*	BQ00590	Q6G1F9	Heat shock protein 70 DnaK	4.6	68.2	42	57	Hsp70	0	Cytoplasmic	9.26	−0.381
JB56	*ftsZ*	BQ08800	O69075	Cell division protein FtsZ homolog	5.1	63.8	35	72	GTPase	5.40E-95	Cytoplasmic	9.12	−0.293
JB57	*typA*	BQ01590	Q6G0R7	GTP-binding protein TypA	5.1	67.2	21	65	GTP binding	1.30E-68	Cytoplasmic	9.97	−0.264
JB59	*fusA*	BQ08260	Q6FZB9	Elongation factor G	4.9	76.6	43	56	GTP binding	1.50E-112	Cytoplasmic	9.97	−0.240
JB60	*fusA*	BQ08260	Q6FZB9	Elongation factor G	4.9	76.6	43	63	GTP binding	1.50E-112	Cytoplasmic	9.97	−0.240
JB61	*pnp*	BQ01980	Q6G0P7	Polyribonucleotide nucleotidyltransferase	5.2	80.8	42	61	RNase	1.00E-47	Cytoplasmic	9.97	−0.233
JB62	*pnp*	BQ01980	Q6G0P7	Polyribonucleotide nucleotidyltransferase	5.2	80.8	37	55	RNase	1.00E-47	Cytoplasmic	9.97	−0.233
JB63	*pnp*	BQ01980	Q6G0P7	Polyribonucleotide nucleotidyltransferase	5.2	80.8	34	66	RNase	1.00E-47	Cytoplasmic	9.97	−0.233
JB64	*ppdK*	BQ03760	Q6G0B6	Pyruvate phosphate kinase	5.3	97.5	35	33	Kinase	1.30E-170	Cytoplasmic	9.97	−0.147
JB65	*ppdK*	BQ03760	Q6G0B6	Pyruvate phosphate kinase	5.3	97.5	45	44	Kinase	1.30E-170	Cytoplasmic	9.97	−0.147
JB66	*clpB*	BQ11170	Q6G134	ATP-dependent Clp protease	5.9	98.4	46	49	ATPase	6.80E-106	Cytoplasmic	9.97	−0.383
JB67	*clpB*	BQ11170	Q6G134	ATP-dependent Clp protease	5.9	98.4	49	71	ATPase	6.80E-106	Cytoplasmic	9.97	−0.383
JB68	*acnA*	BQ01090	Q6G0V8	Aconitate hydratase	6.2	98.2	34	49	Aconitase	5.20E-234	Cytoplasmic	9.26	−0.184
	alaS	BQ07940	Q6FZF1	Alanyl-tRNA synthetase	6.2	98.0	21	35	Synthetase	0	Cytoplasmic	9.97	−0.182
JB69	*acnA*	BQ01090	Q6G0V8	Aconitate hydratase	6.2	98.2	46	39	Aconitase	5.20E-234	Cytoplasmic	9.26	−0.184
JB70	*rho*	BQ13580	Q6FYB7	Transcription terminator factor rho	5.3	47.3	25	60	ATP synthase	4.10E-80	Cytoplasmic	8.96	−0.212
	serS	BQ04770	Q6G036	Seryl tRNA synthetase	5.6	47.8			Synthetase	2.80E-61	Cytoplasmic	10.00	−0.302
JB71	NA	BQ09000	Q6G1K4	ABC transporter, ATP-binding protein	5.8	61.1	32	45	ABC transporter	1.00E-48	Cytoplasmic	7.88	−0.424
JB72	*atpD*	BQ12230	Q6FYM3	ATP synthase beta chain	5.6	57.1	31	58	ATP synthase	2.30E-98	Cytoplasmic	8.96	−0.128
JB73	*guaB*	BQ01690	Q6G181	Inosine-5′-monophosphate dehydrogenase	6.6	52.9	35	55	Dehydrogenase/reductase	1.20E-224	Cytoplasmic	8.96	−0.020
JB74	*mopA*	BQ10750	033964	Chaperonin protein GroEL	4.9	57.6	24	54	Chaperonin	1.10E-194	Cytoplasmic	9.97	−0.041
JB75	*vompB*		Q64HS8	Variable outer membrane protein	5.0	108.8	27	29	Hemagglutinin	8.40E-06	Outer membrane	8.97	−0.372
	vompA		Q64HS9	Variable outer membrane protein	5.0	101.2	26	29	Hemagglutinin	8.40E-06	Outer membrane	10.00	−0.387
	badA3	BQ01410	Q6G0T2	Surface protein/*Bartonella* adhesin	5.2	104.2	18	21	Hemagglutinin	8.20E-06	Outer membrane	9.52	−0.372
JB77	*vompC*		Q64HS7	Variable outer membrane protein	5.2	104.3	22	41	Hemagglutinin	8.40E-06	Outer membrane	9.52	−0.383
	badA3	BQ01410	Q6G0T2	Surface protein/*Bartonella* adhesin	5.2	104.2	20	39	Hemagglutinin	8.20E-06	Outer membrane	9.52	−0.372
	vompA		Q64HS9	Variable outer membrane protein	5.0	101.2	20	41	Hemagglutinin	8.40E-06	Outer membrane	10.00	−0.387
	badA2	BQ01400	Q6G0T3	Variable outer membrane protein	4.8	101.3	19	41	Hemagglutinin	8.40E-06	Outer membrane	10.00	−0.393
	vompB		Q64HS8	Variable outer membrane protein	5.0	108.8	19	38	Hemagglutinin	8.40E-06	Outer membrane	8.97	−0.372
JB78	*mopA*	BQ10750	033964	Chaperonin protein GroEL	4.9	57.6	13	29	Chaperonin	1.10E-194	Cytoplasmic	9.97	−0.041
	keratin			Cytokeratin 9			17	40					
JB79	*tuf1*	BQ08250	Q6FZC0	Elongation factor tu	5.1	42.9	24	57	GTP binding	1.40E-98	Cytoplasmic	9.97	−0.153
JB84	*comL*	BQ08780	Q6FZ79	Competence lipoprotein ComL precursor	9.6	33.9	13	31	FHA domain	0.92	Unknown		−0.434
	hbpE	BQ08430	Q8KP11	Hemin-binding protein E	4.9	33.0	10	30	Porin	0.014	Outer membrane	9.93	−0.190
JB85	NA	BQ11790	Q6FYQ2	Hypothetical protein	5.2	26.8	11	24	Unknown domain	2.40E-134	Unknown		−0.352

Continued on following page

TABLE 2—*Continued*

Spot	Gene	Locus	UniProt accession no.	TIGR protein description	Predicted pI	Predicted mol wt (10^3)	% Mass values matched	Sequence coverage (%)	Pfam model	Pfam E-value[a]	PSORTb localization	PSORTb probability[b]	GRAVY value[c]
JB86	*etfB*	BQ09670	Q6FZ26	Electron transfer flavoprotein beta subunit	6.2	27.1	22	50	Electron transfer flavoprotein	4.90E-68	Cytoplasmic	8.96	0.000
JB87	*hbpA*	BQ02420	Q9KHA7	Hemin-binding protein A	9.5	29.3	19	43	Porin	2.70E-05	Outer membrane	9.45	−0.119
JB88	*hbpD*	BQ04010	Q8KP12	Hemin-binding protein D	9.0	32.7	13	29	Porin	2.00E-14	Outer membrane	10.00	−0.247
JB90	*recA*	BQ07950	Q6FZF0	Recombinase A protein	5.3	37.5	21	54	DNA recombination	2.10E-246	Cytoplasmic	8.96	−0.052
JB91	*omp43*	BQ09890	Q6FZ11	Outer membrane protein	9.5	44.0	30	55	Porin	1.80E-51	Outer membrane	9.93	−0.291
JB92	*nuoD*	BQ05670	Q6FZY8	NADH dehydrogenase I, D subunit	6.8	44.9	28	44	NADH dehydrogenase	2.00E-162	Cytoplasmic	8.96	−0.168
JB93	*glyA*	BQ05390	Q6G009	Serine hydroxymethyltransferase	7.0	47.8	20	32	Hydroxymethyltransferase	4.00E-261	Cytoplasmic	9.97	−0.152
	purB	BQ07450	Q6FZJ4	Adenylosuccinate lyase	6.9	49.8	17	36	Lyase	3.80E-60	Cytoplasmic	9.97	−0.177
JB94	*glyA*	BQ05390	Q6G009	Serine hydroxymethyltransferase	7.0	47.8	24	39	Hydroxymethyltransferase	4.00E-261	Cytoplasmic	9.97	−0.152
	purB	BQ07450	Q6FZJ4	Adenylosuccinate lyase	6.9	49.8	18	33	Lyase	3.80E-60	Cytoplasmic	9.97	−0.162
JB95	*fabB*	BQ01210	Q6G0U6	3-Oxoacyl-(acyl-carrier-protein) synthase I	6.5	43.7	20	44	Synthase	1.20E-54	Cytoplasmic	9.97	−0.147
JB96	*dnaN*	BQ01120	Q6G0V5	DNA polymerase III, beta chain	5.3	41.0	22	53	DNA polymerase III	2.80E-46	Cytoplasmic	8.96	−0.003
JB97	*hs1U*	BQ01960	Q6G0P9	Heat shock protein Hs1U	5.1	48.5	25	31	ATPase	7.00E-43	Cytoplasmic	8.96	−0.179
	lysC	BQ03060	Q6G010	Aspatokinase, alpha and beta subunits	5.1	45.3	15	31	Amino acid kinase	1.50E-69	Unknown		−0.004
JB98	*rho*	BQ13580	Q6FYB7	Transcription terminator factor rho	5.3	47.3	39	52	ATP synthase	4.10E-80	Cytoplasmic	8.96	−0.212
JB101	*sdhA*	BQ12700	Q6FYH8	Succinate dehydrogenase, flavoprotein subunit	5.8	67.0	22	44	Flavin adenine dinucleotide binding	2.40E-175	Cytoplasmic/ periplasmic	5.41/4.48	−0.248
JB102	*rpoB*	BQ07130		DNA-directed RNA polymerase beta chain	4.9	154.8	48	32	RNA polymerase	5.70E-203	Cytoplasmic	9.97	−0.323
JB103	*secA*	BQ01870	Q6G0Q8	Preprotein translocase SecA subunit	5.5	104.4	27	46	ATP binding	1.10E-205	Cytoplasmic	9.12	−0.545
JB104	*carB*	BQ09240	Q6FZ64	Carbamoyl-phosphate synthase large chain	6.6	127.8	37	38	ATP binding	3.60E-113	Cytoplasmic membrane	4.90	−0.157
JB106	*omp89*	BQ06950	Q6G1J3	Outer membrane protein	9.3	88.8	43	41	Surface antigen	1.80E-79	Outer membrane	10.00	−0.261
JB107	*hbpE*	BQ08430	Q8KP11	Hemin-binding protein E	4.9	33.0	27	33	Porin	0.014	Outer membrane	9.93	−0.190
JB108	*tuf1*	BQ08250	Q6FZC0	Elongation factor tu	5.1	42.9	36	43	G-protein superfamily	1.40E-98	Cytoplasmic	9.97	−0.153
JB109	*mopA*	BQ10750	033964	Chaperonin protein GroEL	4.9	57.6	37	50	Chaperonin	1.10E-194	Cytoplasmic	9.97	−0.041
JB113	*hbpE*	BQ08430	Q8KP11	Hemin-binding protein E	4.9	33.0	12	47	Porin	0.014	Outer membrane	9.93	−0.190
JB114	*hbpE*	BQ08430	Q8KP11	Hemin-binding protein E	4.9	33.0	14	29	Porin	0.014	Outer membrane	9.93	−0.190
JB115	*tuf1*	BQ08250	Q6FZC0	Elongation factor tu	5.1	42.9	17	34	G-protein superfamily	1.40E-98	Cytoplasmic	9.97	−0.153
JB116	*vompA*		Q64HS9	Variable outer membrane protein	5.0	101.2	28	33	Hemagglutinin	8.40E-06	Outer membrane	10.00	−0.387
	badA2	BQ01400	Q6G0T3	Variable outer membrane protein	4.8	101.3	26	31	Hemagglutinin	8.40E-06	Outer membrane	10.00	−0.393
	vompC		Q64HS7	Variable outer membrane protein	5.2	104.3	26	31	Hemagglutinin	8.40E-06	Outer membrane	9.52	−0.383
	badA3	BQ01410	Q6G0T2	Surface protein/*Bartonella* adhesin	5.2	104.2	24	28	Hemagglutinin	8.20E-06	Outer membrane	9.52	−0.372
JB117	*vompB*		Q64HS8	Variable outer membrane protein	5.0	108.8	32	44	Hemagglutinin	8.40E-06	Outer membrane	10.00	−0.387
JB118	*tig*	BQ07290	Q6F2K6	Trigger factor	4.7	53.5	34	56	Trigger factor protein	1.40E-52	Cytoplasmic	8.96	−0.565
JB119	*hbpD*	BQ04010	Q8KP12	Hemin-binding protein D	9.0	32.7	27	43	Porin	2.00E-14	Outer membrane	10.00	−0.247
JB120	*vompA*		Q64HS9	Variable outer membrane protein	5.0	101.2	18	39	Hemagglutinin	8.40E-06	Outer membrane	10.00	−0.387
JB121	*mopA*	BQ10750	033964	Chaperonin protein GroEL	4.9	57.6	22	32	Chaperonin	1.10E-194	Cytoplasmic	9.97	−0.041
JB122	*clpB*	BQ11170	Q6G134	ATP-dependent Clp protease	5.9	98.4	24	40	ATPase	6.80E-106	Cytoplasmic	9.97	−0.383
JB123	*badA3*	BQ01410	Q6G0T2	Surface protein/*Bartonella* adhesin	5.2	104.2	19	34	Hemagglutinin	8.20E-06	Outer membrane	9.52	−0.372
	vompC		Q64HS7	Variable outer membrane protein	5.2	104.3	19	33	Hemagglutinin	8.40E-06	Outer membrane	9.52	−0.383
JB124	*omp43*	BQ09890	Q6FZ11	Outer membrane protein	9.5	44.0	26	66	Porin	1.80E-51	Outer membrane	9.93	−0.291
	NA	BQ08370	Q6FZA9	Outer membrane protein	9.9	48.4	16	52	Outer membrane efflux protein	3.70E-32	Outer membrane	10.00	−0.096

JB125	*gyrB*	BQ00370	Q6G119	DNA gyrase subunit B	5.9	89.6	20	23	DNA gyrase B	5.10E-87	Cytoplasmic	8.96	−0.298
JB126	*omp89*	BQ06950	Q6G1J3	Outer membrane protein	9.3	88.8	44	46	Surface antigen	1.80E-79	Outer membrane	10.00	−0.261
JB129	*atpF2*	BQ03160	Q6G0H0	ATP synthase B chain	4.9	18.6	24	47	ATP synthase B/B′ CF(0)	1.70E-16	Unknown		−0.253
JB133	NA	BQ09000	Q6G1K4	ABC transporter, ATP-binding protein	5.8	61.1	29	61	ABC transporter	3.00E-54	Cytoplasmic	7.88	−0.424
JB135	*guaA*	BQ01710	Q6G197	GMP synthase	5.9	57.5	29	38	Glutamine amidotransferase class I	7.20E-46	Cytoplasmic	8.96	−0.021
JB137	*hbpD*	BQ04010	Q8KP12	Hemin-binding protein D	9.0	32.7	31	37	Porin	2.00E-14	Outer membrane	10.00	−0.247

[a] Expectation value. A value of 1 is what is expected by chance (4, 11).
[b] Localization predictions based on PSORTb evaluation. The values are localization probabilities (from 0 to 10) (14).
[c] GRAVY, grand average of hydropathy (23).
[d] NA, not applicable.
[e] JB15, JB16, and JB17 had weak identifications based on PMF; the protein identification was confirmed by performing high-performance liquid chromatography-tandem mass spectrometry.

Twenty-four percent (26/110) of the proteins identified were predicted by PSORTb to localize to the outer membrane, 65% (72/110) of the proteins were predicted to localize to the cytoplasm, and the localizations of 11% (12/110) of the proteins are not known. The 26 proteins localized to the outer membrane correspond to 10 distinct gene products, including three hemin-binding proteins (HbpA, HbpD, and HbpE), Omp43, Omp89, peptidyl-prolyl *cis-trans*-isomerase (Ppi), BQ08370 (a putative OMP), and three adhesins (VompA, VompB, and VompC). The spots with the greatest apparent protein concentration correspond to GroEL (MopA) (at 57.6 kDa; spots JB51, JB52, JB74, JB78, JB109, and JB121), EF-Tu (Tuf1) (at 42.9 kDa; spots JB1/108, JB37, JB38, JB79, and JB115), HbpA (at 29.3 kDa; spot JB87), HbpD (at 32.7 kDa; spots JB2, JB3/31, JB30, JB88, JB119, and JB137), HbpE (at 33 kDa; spots JB4/107, JB5/113, and JB114), VompA (97.0 kDa), VompB (100.5 kDa), and VompC (99.8 kDa). Pfam predictions and grand average of hydropathy values for all of the spots identified are shown in Tables 2 and 3.

Immunoblotting with human sera identified 24 immunoreactive *B. quintana* membrane proteins. We identified 24 *B. quintana* proteins that are recognized consistently by sera from humans with documented *B. quintana* infections. TMP from the same preparation that was used for PMF were separated simultaneously by 2D SDS-PAGE to produce two identical 2D gels, and then TMP from one gel were transferred and immunoblotted with sera from each of 21 patients from whom *B. quintana* was isolated and whose sera were positive for *Bartonella* antibodies as determined by IFA analysis (10, 40) (Table 1). Each patient's serum was analyzed on a separate immunoblot, and immunoreactive antigens were identified by alignment with a simultaneously prepared silver-stained gel using the 2D Evolution software (Nonlinear Dynamics). For negative controls, two blots with 2D-separated TMP were immunoblotted with sera that were from *Bartonella* IFA-negative, culture-negative patients. These control sera detected a few *B. quintana* proteins, usually the protein spots that had the highest protein concentrations and were most dense. The proteins that were immunoreactive on these two negative control blots were considered false positives and were not included in the analysis of positive sera.

To identify the *B. quintana* antigens most commonly recognized by sera from patients infected with *B. quintana*, we established a positive cutoff value of 24, representing the *B. quintana* TMP antigens recognized by sera from 24% or more of the patients infected with *B. quintana* (at least 5 of the 21 patients analyzed). Using this cutoff value, we identified 24 immunodominant *B. quintana* proteins recognized by sera from these patients (Table 4). Figure 3 shows a representative 2D immunoblot of *B. quintana* TMP probed with serum from patient 4 (Table 1). The pI values of these immunoreactive antigens ranged from 4 to 7, and the predicted molecular masses ranged from 20 to 100 kDa. Four of the immunodominant antigens were OMP (VompA, VompB, HbpE, and Ppi). The remainder were predicted to be cytoplasmic proteins, and many of these cytoplasmic proteins have been identified previously in the outer membrane fractions of other gram-negative organisms, including other *Bartonella* species (6, 16, 42). Each of the 24 immunoreactive antigens commonly recognized by patients' sera was labeled in the immunoblot shown in Fig.

TABLE 3. *B. quintana* proteins identified in this study and predicted to be localized to the outer membrane by PSORTb[a]

Gene	Spot	TIGR protein description	Predicted mol wt (10^3)	Predicted pI	% Sequence coverage	PSORTb probability	Pfam model	Pfam E-value	GRAVY value[b]
hbpA	JB87	Hemin-binding protein A	29.3	9.5	43	9.45	Porin	2.70E-05	−0.119
hbpD	JB2	Hemin-binding protein D	32.7	9.0	21	10.00	Transmembrane domain	0.0033	−0.247
hbpD	JB3 (JB31)[c]	Hemin-binding protein D	32.7	9.0	19 (35)	10.00	Transmembrane domain	0.0033	−0.247
hbpD	JB30	Hemin-binding protein D	32.7	9.0	40	10.00	Transmembrane domain	0.0033	−0.247
hbpD	JB88	Hemin-binding protein D	32.7	9.0	29	10.00	Transmembrane domain	0.0033	−0.247
hbpD	JB119	Hemin-binding protein D	32.7	9.0	43	10.00	Transmembrane domain	0.0033	−0.247
hbpE	JB4 (JB107)[c]	Hemin-binding protein E	33.0	4.9	37 (33)	9.93	Porin	0.014	−0.190
hbpE	JB5 (JB113)[c]	Hemin-binding protein E	33.0	4.9	31 (47)	9.93	Porin	0.014	−0.190
hbpE	JB84	Hemin-binding protein E	33.0	4.9	30	9.93	Porin	0.014	−0.190
hbpE	JB114	Hemin-binding protein E	33.0	4.9	29	9.93	Porin	0.014	−0.190
ppi	JB33	Peptidyl-prolyl *cis-trans*-isomerase	35.7	5.6	68	9.92	Rotamase	1.20E-38	−0.415
ppi	JB35	Peptidyl-prolyl *cis-trans*-isomerase	35.7	5.6	57	9.92	Rotamase	1.20E-38	−0.415
omp43	JB42 (JB91)[c]	Outer membrane protein	44.0	9.5	50 (55)	9.93	Porin	1.80E-51	−0.291
omp43	JB124	Outer membrane protein	44.0	9.5	66	9.93	Porin	1.80E-51	−0.291
BQ08370		Outer membrane protein	48.4	9.9	52	10.00	Outer membrane efflux protein	3.70E-32	−0.096
omp89	JB106 (JB126)[c]	Outer membrane protein	88.8	9.3	41 (46)	10.00	Surface antigen	1.80E-79	−0.261
vompA	JB75	Variable outer membrane protein	101.2	5.0	29	10.00	Hemagglutinin	8.40E-06	−0.387
vompB		Variable outer membrane protein	108.8	5.0	29	8.97	Hemagglutinin	8.40E-06	−0.372
badA3		Surface protein/*Bartonella* adhesin	104.2	5.2	21	9.52	Hemagglutinin	8.20E-06	−0.372
vompA	JB77	Variable outer membrane protein	101.2	5.0	41	10.00	Hemagglutinin	8.40E-06	−0.387
vompC		Variable outer membrane protein	104.3	5.2	41	9.52	Hemagglutinin	8.40E-06	−0.383
badA2		Variable outer membrane protein	101.3	4.8	41	10.00	Hemagglutinin	8.40E-06	−0.393
badA3		Surface protein/*Bartonella* adhesin	104.2	5.2	39	9.52	Hemagglutinin	8.20E-06	−0.372
vompB		Variable outer membrane protein	108.8	5.0	38	8.97	Hemagglutinin	8.40E-06	−0.372
vompA	JB116	Variable outer membrane protein	101.2	5.0	33	10.00	Hemagglutinin	8.40E-06	−0.387
badA2		Variable outer membrane protein	101.3	4.8	31	10.00	Hemagglutinin	8.40E-06	−0.393
vompC		Variable outer membrane protein	104.3	5.2	31	9.52	Hemagglutinin	8.40E-06	−0.383
badA3		Surface protein/*Bartonella* adhesin	104.2	5.2	28	9.52	Hemagglutinin	8.20E-06	−0.372
vompA	JB120	Variable outer membrane protein	101.2	5.0	39	10.00	Hemagglutinin	8.40E-06	−0.387
badA3	JB123	Surface protein/*Bartonella* adhesin	104.2	5.2	34	9.52	Hemagglutinin	8.20E-06	−0.372
vompC		Variable outer membrane protein	104.3	5.2	33	9.52	Hemagglutinin	8.40E-06	−0.383
vompB	JB117	Variable outer membrane protein	108.8	5.0	38	8.97	Hemagglutinin	8.40E-06	−0.372

[a] Twenty-one spots representing 10 genes were analyzed.
[b] GRAVY, grand average of hydropathy.
[c] Some protein spots were identified by PMF more than once: the numbers in parentheses are the spot numbers for the duplicate proteins.

3. Note that the serum from *B. quintana*-infected patient 4 recognized all of the 24 immunoreactive proteins whose values were above the 24% cutoff value for all patients. Antibodies in the serum of patient 4 also recognized several *B. quintana* protein spots whose values were below the cutoff value of 24%; therefore, although these proteins (e.g., NusA, VompC, CarB, SecA, SdhA, and PdhB) were strongly immunoreactive on the blot, they were not labeled or included in Table 4. Note that in some cases (e.g., GroEL [MopA], AtpA, EF-Tu [Tuf1], RpsA, HbpE, and Pnp), the most dominant member of a protein family did not meet the inclusion criteria because the spot was so highly concentrated that it was also immunoreactive with the negative sera. Therefore, these proteins were not included in the analysis.

Spots JB15 and JB17 had low sequence coverage as determined by PMF, and spot JB16 had no significant hits in the database search but was found to have monoisotopic peaks similar to those of spots JB15 and JB17. Because these proteins were found to be highly immunogenic, we confirmed their identities by submitting the peptides for mass spectrometry/mass spectrometry analysis. Validation of the liquid chromatography-mass spectrometry/mass spectrometry results confirmed that these three protein spots (spots JB15, JB16, and JB17) were dihydrolipoamide succinyltransferase (SucB) from *B. quintana*.

In addition to the immunoblot analysis, screening of an expression library was performed to identify antigenic proteins using a λ phage genomic library of *B. quintana* JK31. The primary screen included ~3,000 plaques per plate and four plates (a total of 12,000 plaques). Twelve plaques were identified as plaques that were reactive with human and rabbit antisera, and they were replaqued and rescreened to ensure clonality. Characterization of two positive clones resulted in identification of SucB and GroEL (MopA), confirming the immunoreactivities of these two proteins observed by immunoblot analysis.

DISCUSSION

The outer membrane of a bacterium forms the interface between the microorganism and the host and plays an essential role in adhesion and host immune evasion, two important virulence mechanisms utilized by *B. quintana*. The bacterial proteins mediating these interactions between *B. quintana* and its host are critical targets of the host immune response and often have diagnostic relevance, and they are useful candidate antigens for vaccine development. Our goals were to characterize the TMP subproteome of *B. quintana* and to further identify the immunome subset of proteins recognized by sera from humans infected with *B. quintana*. The membrane proteins that we identified are involved in pathogenesis and also are candidate antigens for diagnostic evaluation, treatment, and prevention of *B. quintana* infection, especially in patients with concomitant human immunodeficiency virus infections.

2D mapping of the *B. quintana* TMP fraction by PMF identified 60 individual proteins. One-quarter of the proteins that

TABLE 4. *B. quintana* proteins found to be immunoreactive with sera from patients infected with *Bartonella*

Spot	Gene	Protein description	% of patients reactive[a]	Vol of spot[b]		
				Mean	Median	Range
OMP						
VompB	*vompB*	Variable outer membrane protein B	33	26.0	20.4	5.6–97.1
VompA	*vompA*	Variable outer membrane protein A	29	31.5	22.3	6.0–120.2
JB33	*ppi*	Peptidyl-prolyl *cis-trans*-isomerase	29	8.4	7.5	2.2–17.8
JB114	*hbpE*	Hemin-binding protein E	24	5.2	3.8	1.3–12.5
Non-OMP						
JB16	*sucB*	Dihydrolipoamide succinyltransferase	76	34.0	21.2	3.1–146.8
JB17	*sucB*	Dihydrolipoamide succinyltransferase	76	17.6	9.9	0.5–89.4
JB15	*sucB*	Dihydrolipoamide succinyltransferase	52	20.3	9.8	2.4–101.5
JB12	*guaB*	Inosine-5′-monophosphate dehydrogenase	52	11.8	7.5	2.3–51.2
JB38	*tuf1*	Elongation factor tu	48	7.9	6.1	3.1–18.1
JB51	*mopA*	Chaperonin protein GroEL	48	19.3	13.9	2.3–62.5
JB52	*mopA*	Chaperonin protein GroEL	48	24.1	19.8	1.9–104.0
JB74	*mopA*	Chaperonin protein GroEL	48	9.0	6.5	1.7–29.0
JB14	*atpD*	ATP synthase beta chain	43	15.6	8.8	3.7–50.0
JB37	*tuf1*	Elongation factor tu	43	10.4	7.5	1.7–47.4
JB79	*tuf1*	Elongation factor tu	43	13.8	10.6	2.3–69.3
JB10	*rplY*	50S ribosomal protein L25	38	31.5	22.9	4.6–87.9
JB11	*sucD*	Succinyl-coenzyme A synthetase alpha chain	38	8.5	7.3	4.1–16.2
JB19	*rpsA*	30S ribosomal protein s1	38	5.8	5.5	0.7–11.3
JB65	*ppdK*	Pyruvate phosphate kinase	38	4.4	3.0	0.7–15.2
JB9		Hypothetical protein	33	15.2	10.1	4.5–44.6
JB21	*rpsA*	30S ribosomal protein s1	33	9.5	5.2	1.2–53.5
JB47	*atpD*	ATP synthase beta chain	33	10.6	7.5	4.3–30.2
JB55	*dnaK*	Heat shock protein 70 DnaK	33	28.2	27.2	6.6–72.7
JB109	*mopA*	Chaperonin protein GroEL	33	51.8	39.2	3.5–217.7
JB25	*ibpA2*	Small heat shock protein	29	8.3	6.8	2.3–15.1
JB29	*purC*	Phosphoribosylaminoimidazole-succinocarboxamide synthase	29	4.7	3.8	1.9–8.7
JB45	*atpA*	ATP synthase alpha chain	29	5.6	3.4	1.6–13.3
JB56	*ftsZ*	Cell division protein FtsZ homolog	29	5.7	4.6	1.5–13.8
JB57	*typA*	GTP-binding protein TypA	29	4.3	4.0	2.7–6.9
JB63	*pnp*	Polyribonucleotide nucleotidyltransferase	29	4.1	4.3	1.1–7.1
JB18	*rho*	Transcription terminator factor rho	24	6.0	4.5	0.3–15.5
JB44	*atpA*	ATP synthase alpha chain	24	5.0	3.3	2.5–11.0
JB50	*tig*	Trigger factor	24	16.7	6.4	1.9–57.9
JB73	*guaB*	Inosine-5′-monophosphate dehydrogenase	24	7.2	3.4	2.7–20.1
JB121	*mopA*	Chaperonin protein GroEL	24	4.0	2.7	1.5–7.2
JB133		ABC transporter, ATP-binding protein	24	4.1	3.3	0.8–8.9

[a] Percentage of patient sera found to have immunoreactivity to the protein of the total number of patients ($n = 21$).
[b] The volume of a spot was determined by dividing the pixel intensity by the area of the spot (2D Evolution Analysis software; Nonlinear Dynamics, Durham, NC).

we identified are predicted to be membrane proteins, nearly one-third of the proteins are predicted to be cytoplasmic proteins, and the remainder have unknown localizations. As found in other gram-negative bacteria (6, 16), including *B. henselae* (42), many of the proteins that fractionated with the TMP fraction are not membrane proteins. We performed a search of the *B. quintana* genome in the TIGR database for "membrane proteins" and identified 55 membrane-associated proteins. Of these 55 proteins, 61.8% were found to have a predicted pI of ~9.0 or higher. With our 2D gel system, we were able to resolve proteins with pI values ranging from 4.5 to 9.5, and therefore we identified only a few OMP with a pI near 9.5, including Omp43 (pI 9.5), Omp89 (pI 9.3), and HbpA (pI 9.5). Identification of the more basic membrane proteins in the pI range from 9.0 to 12.0 requires a different method to improve resolution, and indeed, these very basic membrane proteins of gram-negative bacteria are often refractory to fractionation regardless of the method used (42).

Of the cytosolic proteins that fractionated in the 2D TMP fraction, GroEL (MopA) and EF-Tu (Tuf1) are commonly found in membrane preparations of other gram-negative bacteria (2, 41). Two of the proteins that we identified, GroEL and DnaK, are common heat shock proteins that also function as chaperones and thus are often membrane associated (7, 37). Indeed, *Bartonella bacilliformis* has been shown to actively secrete GroEL (30). Other cytosolic proteins, including FusA, TypA, EF-Tu, and Tig, are ribosome-associated proteins that can be membrane associated during the biosynthesis of proteins destined for the periplasm or outer membrane (17). Additional cytoplasmic proteins are associated with the membrane either transiently or while they are functioning as chaperones (7), and thus our detection of these proteins in the membrane fraction is not unexpected or unprecedented.

Comparison of the OMP subproteomes of *B. henselae* and *B. quintana* identified the Vomp as unique to *B. quintana*. By using 2D SDS-PAGE, we identified 19 membrane proteins that were present in both *B. quintana* and *B. henselae* (42), another species of *Bartonella* that infects AIDS patients. Of the

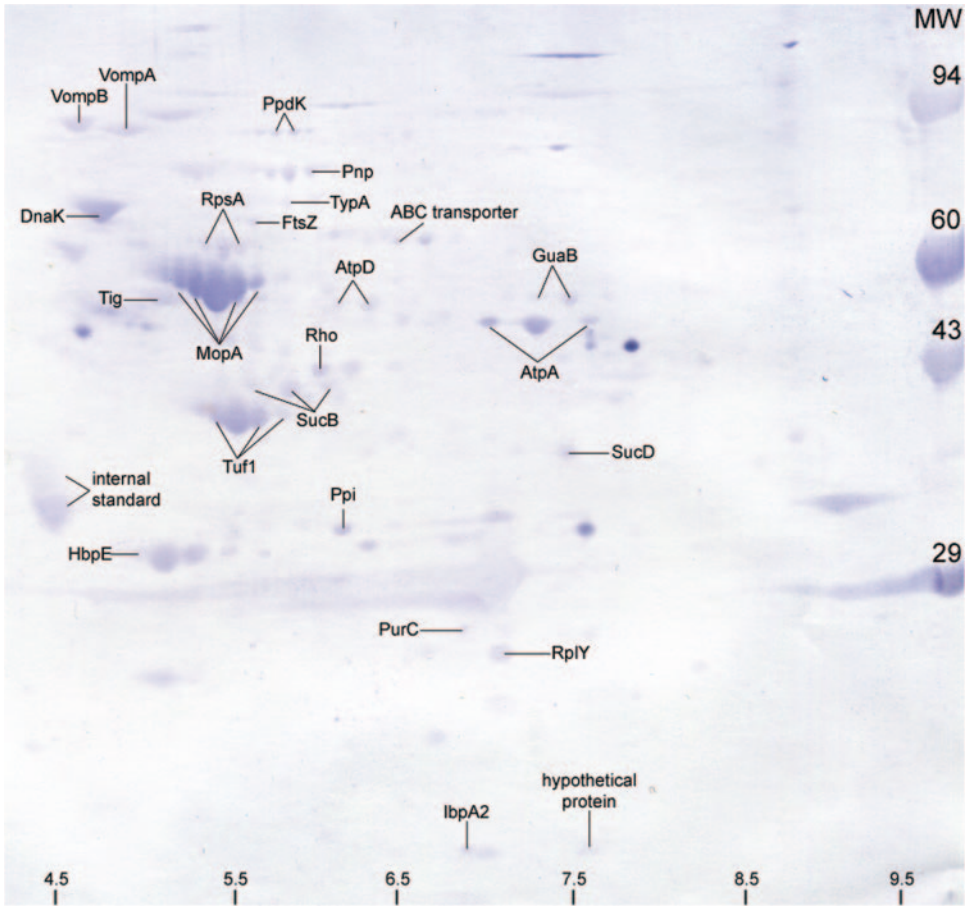

FIG. 3. 2D immunoblot of TMP from *B. quintana* JK31 probed with serum from a *B. quintana*-infected human. 2D separation of the TMP fraction was performed, and the proteins were transferred and immunoblotted with a 1:50 dilution of serum from patient 4, who had a documented *B. quintana* infection. Antibodies from this patient bound all of the antigens which were identified as antigens that were consistently recognized by sera from the 21 patients tested (immunodominant antigens are shown in Table 4). A total of 24 immunodominant antigens were identified; their isoelectric points ranged from 4 to 7, and their molecular masses ranged from 20 to 100 kDa. The positions of molecular weight markers (MW) are indicated on the right, and the pI values are indicated at the bottom.

60 unique membrane-associated *B. quintana* proteins, 13 have characteristics of a prototypical OMP as determined by PSORTb analysis: HbpA, HbpD, HbpE, Omp43, Omp89, Ppi, BQ08370, and six Vomp paralogs. Five of the OMP identified in *B. quintana* have orthologs that also were identified in the sarcosine-insoluble fraction of *B. henselae* (42): HbpA, HbpD, Omp43, Omp89, and Ppi. Two additional OMP were identified in *B. henselae* but not in *B. quintana*: HutA and BH00450. Although the latter two OMP are present in the *B. quintana* genome, they have predicted pI values of 9.5 and 9.9, respectively, and did not resolve well in our system.

Finally, our subproteome analysis identified the following OMP virulence factors that were unique to *B. quintana* and were not found in *B. henselae* (42): VompA, VompB, VompC, and six additional isoforms of Vomp (spots JB75, JB77, JB116, JB117, JB120, and JB123), in addition to HbpE (spots JB4/107, JB5/113, and JB114) (Fig. 2). The *vomp* genes encode a family of four OMP adhesins that contribute to binding of *B. quintana* to collagen and to autoaggregation (45). The Vomp proteins are members of the newly described trimeric autotransporter adhesin family that includes YadA of *Yersinia enterocolitica* (9). Each Vomp has a major variable region near the adhesin tip. The major variable region of each Vomp confers a specific and different virulence phenotype on *B. quintana* (e.g., VompA is necessary and sufficient to mediate autoaggregation). In addition, in a recent study Schulte et al. (44) suggested a specific role for *B. quintana* Vomp in the angiogenic reprogramming of host cells. Infection of human macrophages (THP-1) and epithelial cells (HeLa 229) with *B. quintana* JK31 (a Vomp-expressing strain) induced secretion of vascular endothelial growth factor from both cell types. Strains lacking Vomp expression (BQ2-D70, *B. quintana* Toulouse, and *B. quintana* Munich) did not induce secretion of vascular endothelial growth factor (44). This suggests that the Vomp proteins have a specific pathogenic role in the angiogenesis response that occurs in bacillary angiomatosis lesions.

Five hemin-binding proteins (Hbp) have been described in *B. quintana* (HbpA to HbpE) (29) and are encoded by a five-member gene family comprised of *hbpA* to *hbpE* (29). Five orthologs also occur in *B. henselae* (HbpA to HbpD and Bh10780) (1). We identified three *B. quintana* Hbp by PMF: HbpA (spot JB87), HbpD (spots JB2, JB3/31, JB30, JB88, and

JB119), and HbpE (spots JB4/107, JB5/113, JB84, and JB114) (Fig. 2 and Table 2). Because heme is essential for *B. quintana* (33), it is not surprising to find that Hbp are very abundant OMP. Recent studies have shown that the *hbp* gene family exhibits differential expression in response to environmental cues such as temperature, oxygen, and heme concentration (5), and the *hbpADE* subfamily is markedly induced under conditions that simulate the conditions in the human host. HbpA, HbpD, and HbpE are the most prominent Hbp, and although this is in agreement with previous reports regarding *hbp* gene induction, the pI values of HbpB and HbpC (10.2 and 10.1, respectively) are higher than the highest pI resolved by our system (pI 9.5).

TMP virulence factors, including Vomp and Hbp family members, are highly immunogenic during human *B. quintana* infection. We identified 24 *B. quintana* proteins that are consistently recognized by sera from patients infected with *B. quintana*, using the 2D Evolution software to identify immunoreactive spots, and evaluated spot size relative to intensity. Of these 24 proteins, 4 were proteins that that we identified in this study as OMP: VompA, VompB, HbpE, and Ppi (Table 4 and Fig. 3). Twenty are cytosolic proteins or IMP that also are recognized by sera from patients infected with *B. quintana*. OMP and non-OMP are listed in Table 4 in order of frequency of recognition by human serum.

Of the four immunoreactive OMP that we identified by PMF that are consistently immunoreactive with patient sera, three are known virulence factors: VompA, VompB, and HbpE. The Vomp adhesins are of particular interest because they are unique to *B. quintana* and they play a significant role in virulence during infection (45). *Bartonella* species can survive in the bloodstream for weeks and even months and can adhere to host cell erythrocytes. We demonstrated that the Vomp are surface exposed, using binding of fluorescent antibodies. We found that some *vomp* genes undergo phase variation in vivo and are not expressed during prolonged bloodstream infection (45). It is advantageous for the bacterium to be able to alter the expression of the Vomp adhesins and other virulence-associated factors in order to evade the host immune response. It is therefore noteworthy that the surface-expressed Vomp adhesins are targeted by the human immune system in many patients infected with *B. quintana*, which could be important in generating phase and/or antigenic variation of the *vomp* gene expression in this bacterium. VompC was not recognized by a sufficient number of patients, however, and thus did not meet the criteria for inclusion. It is therefore possible that not all *B. quintana* isolates express all four Vomp proteins, preventing targeting of the Vomp proteins by the host immune system. The lack of antibodies recognizing VompC could be the result of attenuated expression of VompC compared to the expression of VompA and VompB; VompC also may not be comparably immunogenic. Finally, VompC expression could be turned off due to phase variation, before antibodies are elicited in the host. It will be interesting to examine the isolates from the patients whose sera recognized only one or two of the four Vomp proteins to see if a corresponding isolate from a patient has the full complement of four *vomp* genes and, if so, whether the genes are expressed. We used a single strain, *B. quintana* JK31, for antigen preparation, against which we blotted each patient's serum. This enabled us to directly compare the antibody responses of the patients and to determine which antigens are most consistently recognized. However, it is possible that JK31 does not have the same protein profile as the *B. quintana* strain from an individual patient, and this should be investigated further. For instance, from the standpoint of both virulence and diagnosis, it is important to determine if one specific Vomp is always expressed during human infection with *B. quintana*.

HbpE was the only other OMP that was identified as a protein that was immunoreactive with at least 24% of the patient sera. Although differences in gene expression have been noted for *hbpE* (5), more notable is the level of immunogenicity of the HbpE protein compared to the levels of immunogenicity of other members of the Hbp gene family. It should be interesting to further evaluate differences in this protein and to determine the specific role of HbpE in *Bartonella* pathogenesis.

Characterized *B. quintana* immunome includes antigens that have potential diagnostic and vaccine utility. One of our goals was to identify relevant *B. quintana* antigens recognized by the human immune response during the natural course of infection which could lead to both improved diagnosis and an understanding of *Bartonella* infections. Antibody detection is the most widely used diagnostic test for *B. quintana* infection; an IFA test is the current reference method. However, IFA tests are performed in only a few laboratories, and the *Bartonella* IFA test is subjective and extremely laborious. Antigen must be prepared by cocultivation of *Bartonella* with Vero cells on slides, the serum must be serially diluted, and the assay results must be manually screened and graded by highly trained personnel. In addition to the difficulty in performing the IFA test, cross-reactions with other *Bartonella* species can occur. As shown in Table 1, it is apparent that the IFA titers for both *B. henselae* and *B. quintana* are positive in nearly all patients with a documented *B. quintana* infection, and in some cases the titers for the two species are nearly the same, preventing identification of the infecting *Bartonella* strain to the species level. In addition, cross-reactivity can occur with *Coxiella burnetii* and *Chlamydia* species (25, 28). Culture-based diagnosis of *Bartonella* infection is even more difficult and time-consuming (21, 24), and molecular biology techniques have little practical application outside the research lab. In most of the immunoscreens for diagnostic antigens in *Bartonella* workers have used pooled sera from a small number of patients without culture-proven infections or sera collected from experimentally infected small animals (8, 13).

In this study, we utilized a large collection of sera from *B. quintana* culture-positive humans in conjunction with 2D SDS-PAGE and PMF subproteome data for a virulent strain to systematically characterize the total membrane immunome of *B. quintana*, and we identified proteins that are recognized during human infection. All 21 patients had naturally acquired *B. quintana* infections, as documented by isolation of the bacterium from blood or tissue or both (21, 22) and by positive reciprocal IFA titers for *B. quintana* of $\geq$64 (10, 40) (Table 1). Using these sera, 44 immunoreactive *B. quintana* TMP were identified by 2D immunoblot analysis. In addition to these immunodominant TMP, we also found 20 non-TMP that were reactive with one-quarter of the patient sera. We found that SucB (spots JB15, JB16, and JB17) had the highest frequency

of recognition, and we also identified SucB by immunoscreening of an expression library. SucB (dihydrolipoamide succinyltransferase), a 43.8-kDa cytosolic protein, was recognized by 76% of our patient sera (Table 4) and has been detected in immunoscreens of genomic expression libraries for both *B. henselae* and *Bartonella vinsonii* subsp. *berkhoffii* (15, 18, 26). Another cytosolic protein, the 63.8-kDa protein FtsZ (spots JB48 and JB56), reacted with sera from 24% of our patients and has been identified previously as a potential diagnostic antigen for *Bartonella* infection (19, 20, 36). However, because both these cytosolic proteins are highly conserved among bacteria, they are unlikely to be useful for *Bartonella*-specific diagnosis. Considering the frequent recognition and the unique presence of the Vomp and Hbp in *Bartonella*, these antigens are likely to be the most useful antigens for diagnosis of *B. quintana* infections in humans.

In summary, we established a 2D map of the total membrane subproteome of *B. quintana*. We identified 60 unique *B. quintana* proteins by 2D gel electrophoresis and PMF, including OMP virulence factors. Using this newly constructed subproteome map, we identified 24 immunodominant antigens after performing 2D immunoblotting with sera from 21 naturally infected patients. Our goal was to perform a general screen for *B. quintana* antigens that reacted with serum from 24% or more of the patients, as a prelude to more definitive future testing of proteins that appear to be candidate diagnostic and/or vaccine antigens. Additionally, characterization of the *B. quintana* immunome demonstrated that the Vomp virulence factors are frequently recognized by the host immune system, supporting the hypothesis that anti-Vomp antibodies can stimulate the phase variation that occurs in vivo. The identification and evaluation of these *B. quintana* proteins should not only aid in the development of better diagnostic tests and better disease prevention but also provide insight into the pathogenesis of *Bartonella*.

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REFERENCES

1. **Alsmark, C. M., A. C. Frank, E. O. Karlberg, B. A. Legault, D. H. Ardell, B. Canback, A. S. Eriksson, A. K. Naslund, S. A. Handley, M. Huvet, B. La Scola, M. Holmberg, and S. G. Andersson.** 2004. The louse-borne human pathogen Bartonella quintana is a genomic derivative of the zoonotic agent Bartonella henselae. Proc. Natl. Acad. Sci. USA **101:**9716–9721.
2. **Backert, S., T. Kwok, M. Schmid, M. Selbach, S. Moese, R. M. Peek, Jr., W. Konig, T. F. Meyer, and P. R. Jungblut.** 2005. Subproteomes of soluble and structure-bound Helicobacter pylori proteins analyzed by two-dimensional gel electrophoresis and mass spectrometry. Proteomics **5:**1331–1345.
3. **Baik, S. C., K. M. Kim, S. M. Song, D. S. Kim, J. S. Jun, S. G. Lee, J. Y. Song, J. U. Park, H. L. Kang, W. K. Lee, M. J. Cho, H. S. Youn, G. H. Ko, and K. H. Rhee.** 2004. Proteomic analysis of the sarcosine-insoluble outer membrane fraction of *Helicobacter pylori* strain 26695. J. Bacteriol. **186:**949–955.
4. **Bateman, A., L. Coin, R. Durbin, R. D. Finn, V. Hollich, S. Griffiths-Jones, A. Khanna, M. Marshall, S. Moxon, E. L. Sonnhammer, D. J. Studholme, C. Yeats, and S. R. Eddy.** 2004. The Pfam protein families database. Nucleic Acids Res. **32:**D138–D141.
5. **Battisti, J. M., K. N. Sappington, L. S. Smitherman, N. L. Parrow, and M. F. Minnick.** 2006. Environmental signals generate a differential and coordinated expression of the heme receptor gene family of *Bartonella quintana*. Infect. Immun. **74:**3251–3261.
6. **Boyce, J. D., P. A. Cullen, V. Nguyen, I. Wilkie, and B. Adler.** 2006. Analysis of the Pasteurella multocida outer membrane sub-proteome and its response to the in vivo environment of the natural host. Proteomics **6:**870–880.
7. **Caldas, T. D., A. El Yaagoubi, and G. Richarme.** 1998. Chaperone properties of bacterial elongation factor EF-Tu. J. Biol. Chem. **273:**11478–11482.
8. **Chenoweth, M. R., C. E. Greene, D. C. Krause, and F. C. Gherardini.** 2004. Predominant outer membrane antigens of *Bartonella henselae*. Infect. Immun. **72:**3097–3105.
9. **Cotter, S. E., N. K. Surana, and J. W. St. Geme, III.** 2005. Trimeric autotransporters: a distinct subfamily of autotransporter proteins. Trends Microbiol. **13:**199–205.
10. **Dalton, M. J., L. E. Robinson, J. Cooper, R. L. Regnery, J. G. Olson, and J. E. Childs.** 1995. Use of Bartonella antigens for serologic diagnosis of cat-scratch disease at a national referral center. Arch. Intern. Med. **155:**1670–1676.
11. **Finn, R. D., J. Mistry, B. Schuster-Bockler, S. Griffiths-Jones, V. Hollich, T. Lassmann, S. Moxon, M. Marshall, A. Khanna, R. Durbin, S. R. Eddy, E. L. Sonnhammer, and A. Bateman.** 2006. Pfam: clans, web tools and services. Nucleic Acids Res. **34:**D247–D251.
12. **Foucault, C., K. Barrau, P. Brouqui, and D. Raoult.** 2002. Bartonella quintana bacteremia among homeless people. Clin. Infect. Dis. **35:**684–689.
13. **Freeland, R. L., D. T. Scholl, K. R. Rohde, L. J. Shelton, and K. L. O'Reilly.** 1999. Identification of Bartonella-specific immunodominant antigens recognized by the feline humoral immune system. Clin. Diagn. Lab Immunol. **6:**558–566.
14. **Gardy, J. L., M. R. Laird, F. Chen, S. Rey, C. J. Walsh, M. Ester, and F. S. Brinkman.** 2005. PSORTb v. 2.0: expanded prediction of bacterial protein subcellular localization and insights gained from comparative proteome analysis. Bioinformatics **21:**617–623.
15. **Gilmore, R. D., Jr., A. M. Carpio, M. Y. Kosoy, and K. L. Gage.** 2003. Molecular characterization of the *sucB* gene encoding the immunogenic dihydrolipoamide succinyltransferase protein of *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella quintana*. Infect. Immun. **71:**4818–4822.
16. **Haas, G., G. Karaali, K. Ebermayer, W. G. Metzger, S. Lamer, U. Zimny-Arndt, S. Diescher, U. B. Goebel, K. Vogt, A. B. Roznowski, B. J. Wiedenmann, T. F. Meyer, T. Aebischer, and P. R. Jungblut.** 2002. Immunoproteomics of Helicobacter pylori infection and relation to gastric disease. Proteomics **2:**313–324.
17. **Herskovits, A. A., E. Shimoni, A. Minsky, and E. Bibi.** 2002. Accumulation of endoplasmic membranes and novel membrane-bound ribosome-signal recognition particle receptor complexes in Escherichia coli. J. Cell Biol. **159:**403–410.
18. **Kabeya, H., S. Maruyama, K. Hirano, and T. Mikami.** 2003. Cloning and expression of Bartonella henselae sucB gene encoding an immunogenic dihydrolipoamide succinyltransferase homologous protein. Microbiol. Immunol. **47:**571–576.
19. **Kelly, T. M., I. Padmalayam, and B. R. Baumstark.** 1998. Use of the cell division protein FtsZ as a means of differentiating among Bartonella species. Clin. Diagn. Lab Immunol. **5:**766–772.
20. **Knobloch, J.** 1988. Analysis and preparation of Bartonella bacilliformis antigens. Am. J. Trop. Med. Hyg. **39:**173–178.
21. **Koehler, J. E., M. A. Sanchez, C. S. Garrido, M. J. Whitfeld, F. M. Chen, T. G. Berger, M. C. Rodriguez-Barradas, P. E. LeBoit, and J. W. Tappero.** 1997. Molecular epidemiology of bartonella infections in patients with bacillary angiomatosis-peliosis. N. Engl. J. Med. **337:**1876–1883.
22. **Koehler, J. E., M. A. Sanchez, S. Tye, C. S. Garrido-Rowland, F. M. Chen, T. Maurer, J. L. Cooper, J. G. Olson, A. L. Reingold, W. K. Hadley, R. R. Regnery, and J. W. Tappero.** 2003. Prevalence of Bartonella infection among human immunodeficiency virus-infected patients with fever. Clin. Infect. Dis. **37:**559–566.
23. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157:**105–132.
24. **La Scola, B., and D. Raoult.** 1999. Culture of *Bartonella quintana* and *Bartonella henselae* from human samples: a 5-year experience (1993 to 1998). J. Clin. Microbiol. **37:**1899–1905.
25. **La Scola, B., and D. Raoult.** 1996. Serological cross-reactions between *Bartonella quintana*, *Bartonella henselae*, and *Coxiella burnetii*. J. Clin. Microbiol. **34:**2270–2274.
26. **Litwin, C. M., J. M. Johnson, and T. B. Martins.** 2004. The Bartonella henselae sucB gene encodes a dihydrolipoamide succinyltransferase protein reactive with sera from patients with cat-scratch disease. J. Med. Microbiol. **53:**1221–1227.
27. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
28. **Maurin, M., F. Eb, J. Etienne, and D. Raoult.** 1997. Serological cross-reactions between *Bartonella* and *Chlamydia* species: implications for diagnosis. J. Clin. Microbiol. **35:**2283–2287.

29. **Minnick, M. F., K. N. Sappington, L. S. Smitherman, S. G. Andersson, O. Karlberg, and J. A. Carroll.** 2003. Five-member gene family of *Bartonella quintana*. Infect. Immun. **71:**814–821.
30. **Minnick, M. F., L. S. Smitherman, and D. S. Samuels.** 2003. Mitogenic effect of *Bartonella bacilliformis* on human vascular endothelial cells and involvement of GroEL. Infect. Immun. **71:**6933–6942.
31. **Morton, R. J., K. R. Simons, and A. W. Confer.** 1996. Major outer membrane proteins of Pasteurella haemolytica serovars 1-15: comparison of separation techniques and surface-exposed proteins on selected serovars. Vet. Microbiol. **51:**319–330.
32. **Murphy, T. F., K. C. Dudas, J. M. Mylotte, and M. A. Apicella.** 1983. A subtyping system for nontypable Haemophilus influenzae based on outer-membrane proteins. J. Infect. Dis. **147:**838–846.
33. **Myers, W. F., J. V. Osterman, and C. L. Wisseman, Jr.** 1972. Nutritional studies of *Rickettsia quintana*: nature of the hematin requirement. J. Bacteriol. **109:**89–95.
34. **O'Connell, K. L., and J. T. Stults.** 1997. Identification of mouse liver proteins on two-dimensional electrophoresis gels by matrix-assisted laser desorption/ionization mass spectrometry of in situ enzymatic digests. Electrophoresis **18:**349–359.
35. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250:**4007–4021.
36. **Padmalayam, I., B. Anderson, M. Kron, T. Kelly, and B. Baumstark.** 1997. The 75-kilodalton antigen of *Bartonella bacilliformis* is a structural homolog of the cell division protein FtsZ. J. Bacteriol. **179:**4545–4552.
37. **Qi, H. Y., J. B. Hyndman, and H. D. Bernstein.** 2002. DnaK promotes the selective export of outer membrane protein precursors in SecA-deficient Escherichia coli. J. Biol. Chem. **277:**51077–51083.
38. **Raoult, D., and V. Roux.** 1999. The body louse as a vector of reemerging human diseases. Clin. Infect. Dis. **29:**888–911.
39. **Ravaoarinoro, M., C. Ciurli, E. Toma, and R. Morisset.** 1994. Rapid method for isolating detergent-insoluble outer membrane proteins from Pseudomonas aeruginosa. Electrophoresis **15:**594–596.
40. **Regnery, R. L., J. G. Olson, B. A. Perkins, and W. Bibb.** 1992. Serological response to "Rochalimaea henselae" antigen in suspected cat-scratch disease. Lancet **339:**1443–1445.
41. **Renesto, P., S. Azza, A. Dolla, P. Fourquet, G. Vestris, J. P. Gorvel, and D. Raoult.** 2005. Proteome analysis of Rickettsia conorii by two-dimensional gel electrophoresis coupled with mass spectrometry. FEMS Microbiol. Lett. **245:**231–238.
42. **Rhomberg, T. A., O. Karlberg, T. Mini, U. Zimny-Arndt, U. Wickenberg, M. Rottgen, P. R. Jungblut, P. Jeno, S. G. Andersson, and C. Dehio.** 2004. Proteomic analysis of the sarcosine-insoluble outer membrane fraction of the bacterial pathogen Bartonella henselae. Proteomics **4:**3021–3033.
43. **Scherer, D. C., I. DeBuron-Connors, and M. F. Minnick.** 1993. Characterization of *Bartonella bacilliformis* flagella and effect of antiflagellin antibodies on invasion of human erythrocytes. Infect. Immun. **61:**4962–4971.
44. **Schulte, B., D. Linke, S. Klumpp, M. Schaller, T. Riess, I. B. Autenrieth, and V. A. Kempf.** 2006. *Bartonella quintana* variably expressed outer membrane proteins mediate vascular endothelial growth factor secretion but not host cell adherence. Infect. Immun. **74:**5003–5013.
45. **Zhang, P., B. B. Chomel, M. K. Schau, J. S. Goo, S. Droz, K. L. Kelminson, S. S. George, N. W. Lerche, and J. E. Koehler.** 2004. A family of variably expressed outer-membrane proteins (Vomp) mediates adhesion and autoaggregation in Bartonella quintana. Proc. Natl. Acad. Sci. USA **101:**13630–13635.

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