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Genotypic Characteristics of Two Serotypes of *Bartonella henselae*

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The study of 16S rRNA gene sequences of all isolates of *Bartonella henselae* obtained in our laboratory and others from human patients or cats has revealed two genotypes according to the sequence of the 16S rRNA gene. Two isolates of these genotypes have previously been related to two different serotypes, and lack of cross-protection of the two serotypes has been demonstrated in cats. We investigated the grouping of eight strains of *B. henselae* on the basis of 16S ribosomal DNA, 35-kDa protein, Pap 31 protein, and internal transcribed spacer (ITS) gene sequencing; sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles; and monoclonal antibody reactivity studies. Houston-1, 90-615, and SA2 strains showed the same patterns in SDS-PAGE, but they differed from the patterns of *B. henselae* isolates URBHLLY8, URBH LIE9, Cat6, Fizz, and CAL-1. Nine monoclonal antibodies derived from BALB/c mice immunized with *B. henselae* Houston-1 strain reacted only with strains Houston-1, 90-615, and SA2, and not with any other *Bartonella* strains. The two serogroups corresponded with two genotypes based on differences in the sequences of the genes encoding 16S rRNA, 35-kDa protein, and Pap 31 protein. Sequences of ITS genes were highly divergent among strains, as each had a unique sequence and the subdivision was not supported by DNA-DNA relatedness study. Study of 22 additional strains of *B. henselae* isolated from French bacteremic cats demonstrated that they all belong to one or the other of the proposed serotype or genotype.

Bartonella henselae is a gram-negative, oxidase-negative, fastidious, aerobic, rod-shaped, slow-growing bacterium. Stoler et al. (41) first demonstrated the involvement of a bacterium in the etiology of an AIDS-related syndrome, bacillary angiomatosis (BA). In 1990, a 16S ribosomal gene fragment was amplified by PCR directly from tissue samples from patients with BA. By 16S rRNA gene sequencing, the bacterium was identified as *Rochalimaea* (*Bartonella*) spp.-like (33), and the novel species name, *B. henselae*, was proposed in 1992 (32). Improvements in the techniques used to isolate *B. henselae* and new methods for the identification and detection of the organism have enabled further clinical manifestations of infections to be determined. These now include cat scratch disease (CSD) (10, 15, 21, 38), BA (33, 40), peliosis hepatitis (40), septicemia (39), endocarditis (13, 31), and neurological disorders (27).

In 1996, Drancourt et al. reported a new serotype of *B. henselae* named Marseille (10), which was also a new genotype. The authors found that two isolates of *B. henselae*, from a patient with endocarditis and a patient with CSD, were genetically different from all previously isolated strains by sequence analysis of the 16S rRNA-encoding gene. Investigators in The Netherlands (2) also demonstrated two restriction fragment length polymorphism (RFLP) patterns of *B. henselae* DNA in samples from CSD patients. This was shown by analysis of the 16S-23S rRNA gene spacer PCR fragments and 16S rRNA gene PCR products digested with *Alu*I. The presence of two genotypes was later confirmed in France and Germany (17, 19, 37, 38) based on sequencing of the 16S rRNA-encoding gene. Based on 16S RNA gene differences, genotypes I and II were proposed. PCR-based RFLP analysis of the 16S-23S rRNA intergenic spacer region using *Alu*I and *Hae*III demonstrated seven composite RFLP types in 11 *B. henselae* isolates from patients with BA, septicemia, and parenchymal bacillary peliosis (26). Rodriguez-Barradas et al. (34) identified five different fingerprint profiles from 17 isolates of *B. henselae* isolated from cats and from tissue and blood of human immunodeficiency virus-infected patients and patients with CSD. This was demonstrated by repetitive extragenic palindromic PCR and enterobacterial repetitive intergenic consensus PCR. The intergenic spacer region between the 16S and 23S rRNA genes showed that each tested strain (34) had a specific sequence. This method therefore appears to be of limited use in the identification of isolates at the species level. Other tested genes did not produce such diverse results: for example, the sequences of all tested strains of *B. henselae* based on partial citrate synthase gene were identical (4), and comparison of sequences from the riboflavin synthesis proteins of six isolates of *B. henselae* found single-nucleotide differences in some strains but no real difference between the representatives of genotype I and II (1).

In order to better define the classification of *B. henselae* isolates, especially serotypes associated within the two genotypes we observed by 16S rRNA gene sequence determination, we studied protein profiles of the organisms and sequenced additional genes. This was achieved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western immunoblotting with monoclonal antibodies (MAbs) against *B. henselae* Houston-1, and amplification and sequencing of genes that encode for the 35-kDa protein (unpublished data, GenBank access number U21304), the Pap 31 protein of phage 60457 (5) of *B. henselae*, and the internal transcribed spacer (ITS) (35, 36). The results of these studies are described in this report.

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TABLE 1. Sources of *Bartonella* isolates used in this study[a]

Species	Strains	Source (reference)
B. henselae	Houston-1^T (ATCC 4988)	Septicemia, United States (32)
B. henselae	Cat6	Blood from bacteremic cat, South Africa
B. henselae	Fizz	Blood from bacteremic cat, Switzerland
B. henselae	90-615 (King)	BA, United States (45)
B. henselae	SA2	CSD, United States (9)
B. henselae	CAL-1	Septicemia, United States
B. henselae	URBHLLY8-Marseille (CIP 104756)	CSD, France (10)
B. henselae	URBHLIE9	Endocarditis, France (10)
B. quintana	URBQTBAAH1	BA, France (14)
B. quintana	URBQMLY15	Chronic lymphadenopathy, France (11)
B. quintana	URBQPBAA7	BA, France
B. quintana	URBQLIEH6	Endocarditis, France (24)
B. quintana	URBQMTF14	Trench fever, France (7)
B. quintana	URBQMTF15	Trench fever, France (7)
B. quintana	Fuller^T (ATCC VR-358)	Trench fever (42)
B. quintana	URBQLY4	Chronic lymphadenopathy, France[b]
B. quintana	Oklahoma	Septicemia, United States
B. quintana	URBQPIEH2	Endocarditis, France (24)
B. quintana	SH-PERM	NA[c], Russia
B. elizabethae	F9251^T (ATCC 49927)	Endocarditis (8)
B. grahamii	V2^T (NTCC 12860)	Blood from *Clethrionomys glareolus* (3)
B. taylorii	M6^T (NTCC 12861)	Blood from *Apodemus* sp. (3)
B. doshiae	R18^T (NTCC 12862)	Blood from *Microtus agrestis* (3)
B. vinsonii	Baker^T (ATCC VR-152)	Spleen from *Microtus pennsylvanicus* (44)
B. bacilliformis	Acoch 812	Blood from bartonellosis patient, Peru
B. bacilliformis	Monzon 269	Blood from bartonellosis patient, Peru
B. weissii	BW137	Blood from cow, France
B. clarridgeiae	URBCMNHC26	Blood from cat, France
B. koehlerae	C-29 (ATCC 700693)	Blood from cat (12)
Bartonella sp.	C1-phy	Blood from *Phyllotis* sp. (4)
Bartonella sp.	C4-phy	Blood from *Phyllotis* sp. (4)
Bartonella sp.	C5-rat	Blood from *Rattus* sp. (4)
Bartonella sp.	C7-rat	Blood from *Rattus* sp. (4)
Bartonella sp.	C1-phy1	Blood from *Phyllotis* sp. (4)
Bartonella sp.	C1-phy2	Blood from *Phyllotis* sp. (4)
Bartonella sp.	N40	Blood from *Apodemus sylvaticus* (4)

[a] Source is given when isolation of strain was not detailed elsewhere.
[b] Reference: D. Raoult, M. Drancourt, A. Carta, and J. A. Gastaut, Letter, Lancet **343:**977, 1994.
[c] NA, not available.

MATERIALS AND METHODS

***Bartonella* strains and antigen preparations.** The sources of strains used in this study are presented in Table 1. *Bartonella* isolates were grown on Columbia sheep blood agar (BioMerieux, Marcy l'Etoile, France) at 37°C in a 5% carbon dioxide incubator, except for *B. bacilliformis*, which was grown at 32°C. Bacteria were harvested after 5 to 7 days of culture and suspended in deionized water for SDS-PAGE or in phosphate-buffered saline (PBS) for microimmunofluorescence (MIF) assay. Twenty-two additional strains isolated from bacteremic cats, cultivated as described above (23), were used in an MIF assay and for PCR amplification.

Production of MAbs (18). Six-week-old female BALB/c mice were inoculated three times intraperitoneally with 2×10^4 *B. henselae* Houston-1 organisms suspended in 0.5 ml of PBS at 7-day intervals. One week after the third injection, the mice were given intravenous boosters with 4×10^3 organisms suspended in 0.1 ml of PBS. Three days later, spleen cells from immunized mice were subjected to fusion with SP2/0-Ag14 myeloma cells (10:1) using 50% polyethylene glycol (molecular weight, 1,300 to 1,600; Sigma Chemical Co., St. Louis, Mo.) Fusion cells were grown in hybridoma medium (Seromed, Berlin, Germany) with 20% fetal bovine serum (GibcoBRL, Gaithersburg, Md.) and hypoxanthine aminopterin-thymidine (HAT) selective medium (Sigma Chemical Co., St. Louis, Mo.) at 37°C in a humidified atmosphere supplemented with 5% CO_2. The supernatants were screened for antibodies to *B. henselae* by MIF assay. Representative hybridomas were subcloned twice by limiting dilution. Isotypes of MAbs were determined with an ImmunoType Mouse Monoclonal Antibody Isotyping Kit with antisera to mouse immunoglobulin M (IgM), IgA, IgG1, IgG2a, IgG2b, and IgG3 (Sigma). Specificities of MAbs were tested by Western immunoblotting (see below). Ascitic fluids were produced by injecting about 3×10^6 hybridoma cells suspended in 0.5 ml of PBS into mice, 1 week after an intraperitoneal injection of 0.5 ml of 2,6,10,14-tetramethylpentadecane (Pristane; Sigma).

MIF assay. MIF (30) was used to screen hybridoma clones and to determine the specificity of MAbs. Antigens, including all *Bartonella* species listed in Table 1, were placed on 24-well microscope slides with a pen nib. The antigens were fixed in methanol for 10 min at room temperature before MAbs were added at a 1/32 dilution and the slides were incubated in a humidified chamber at 37°C for 30 min. Following two washes in PBS (5 min each) and rinsing with distilled water, the slides were air dried at 37°C. After incubation at 37°C for 30 min with fluorescein (dechlorotriazinyl amino fluorescein)-conjugated goat anti-mouse IgG+IgM (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.), diluted 1:200 in PBS containing 0.2% Evans blue (BioMerieux), the slides were washed (as above), mounted with coverslips using Fluoprep (BioMerieux) and read on a Zeiss epifluorescent microscope (Axioskop20; Carl Zeiss, Gottingen, Germany) at a magnification of ×400. For MAbs that were positive at the 1/32 dilution, MIF titers were determined by end point dilution. Sera from immunized mice were used as positive controls, and sera from healthy mice were used as negative controls. A MAb obtained against *B. henselae* Houston-1 was later used to test 22 *B. henselae* strains isolated from cat blood. MIF assay using a MAb (Bh1H8) diluted 1:200 was performed as described above.

SDS-PAGE and Western immunoblotting. SDS-PAGE (22) and Western immunoblotting were performed using the methods described previously. Equal volumes of antigen (titrated to 4 mg of protein/ml) and sample buffer (0.0625 M Tris hydrochloride [pH 8.0], 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.02% bromophenol blue) were separated electrophoretically in a 12% resolving gel and a 5% stacking gel at a constant current (8 to 10 mA) at room temperature for 4 h in running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS) in an electrophoretic cell (Mini Protein II; Bio-Rad, Richmond, Calif.). Prestained

TABLE 2. List of primers used in this study

Protein encoded by gene	Primer	Nucleotide sequence (5′-3′)
16S rRNA	fD1[a]	AGAGTTGATCCTGGCTCAG
	Rp2[a]	ACGGCTACCTTGTTAGGACTT
	357f	TACGGGAGGCAGCAG
	357r[a]	CTGCTGCCTCCCGTA
	536F	CAGCAGCCGCGGTAATAC
	536R	GTATTACCGCGGCTGCTG
	800F	ATTAGATACCCTGGTAG
	800R	CTACCAGGGTATCTAAT
	1050F	TGTCGTCAGCTCGTG
	1050R	CACGAGCTGACGACA
35 kDa	35KD1f[a]	GTCGCTAAAGGCTGATGA
	35KD2r[a]	GACTGATATCGTGCGTGTG
	35KDs1f	GGTACGACGACAGTAATTGTT
	35KDs2r	GATTTAAGAGATACCAACCA
Pap 31	PAP1f[a]	CTTTAATGACGACTTCTGTT
	PAP4r[a]	CCGAAATCTGAGTAACGGTA
	PAP2r	CCCTAAATGTTTCAAGTTCA
	PAP3f	GCTGACAGAGAAGACGCAA
ITS	16SF[a]	AGAGGCAGGCAACCACGGTA
	23S1[a]	GCCAAGGCATCCACC
	QHVE1	TTGGGATCATCATCTGAA
	QHVE2	TTGGGATCATCATCTGAA
	QHVE3	GATATATTCAGACATGTT
	QHVE4	AACATGTCTGAATATATC

[a] Primer used for PCR amplification and sequencing.

SDS-PAGE standards (Low-Range Standard; Bio-Rad) were used as a reference. The separated antigens on the gels were transferred to a 0.45-μm-pore-size nitrocellulose membrane (Hybond-C; Amersham, Little Chalfont, United Kingdom) at 50 V for 1 h at 4°C in an electrophoretic transfer cell (Mini Trans-Blot; Bio-Rad) and incubated overnight in PBS with 5% nonfat dry milk to block nonspecific binding sites. The membranes were washed three times with PBS, air dried, and cut into strips, which were incubated with MAbs, diluted 1:10 in PBS containing 3% milk, at room temperature for 1 h and washed as described above. Bound antibodies were detected with peroxidase-conjugated $F(ab')_2$ fragment goat anti-mouse IgG (heavy and light chains; AffiniPure; Jackson ImmunoResearch) and diluted at 1:200 in PBS containing 3% nonfat dry milk at room temperature for 1 h. After washing in PBS (as above), color was developed by the addition of a solution containing 0.015% 4-chloro-1 naphthol (Sigma, St. Louis, Mo.), 0.015% hydrogen peroxide, and 16.7% methanol in Tris-buffered saline.

DNA-DNA relatedness. DNA was extracted and purified as previously described (6). The procedure for labeling DNA with tritium-labeled nucleotides and for hybridization experiments (S1 nuclease treatment, trichloroacetic procedure) have been detailed elsewhere (16, 20).

Gene amplification. PCR was carried out in a Peltier model PTC-200 thermal cycler (MJ Research, Inc.). Five microliters of the purified genomic DNA was amplified in 50-μl reaction volumes with the different amplification primers (Table 2) (43). Genes encoding 16S rRNA, 35-kDa protein, Pap 31 protein, and ITS were amplified by using, respectively, fD1-rP2, 35KD1f-35KD1r, PAP1f-PAP4r, and QHVE1-QHVE2 primer pairs. Reaction mixture contained 25 pmol of each primer, 2 U of *Taq* DNA polymerase (GibcoBRL), a 0.2 mM concentration of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, and dTTP), 1.6 μl of $MgCl_2$ (50 mM), and 5 μl of template DNA, with a final volume of 50 μl. For amplification, a 10-min predenaturation step at 95°C was followed by 40 cycles consisting of denaturation at 95°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 1 min 30 s. Amplification was completed by incubation for 3 min at 72°C to allow complete extension of the PCR products. For study of the gene encoding 16S rRNA of the 22 additional *B. henselae* strains isolated from bacteremic cats, PCR incorporated the fD1-357f primer pair under conditions described above.

Purification and sequence analysis of PCR products. The PCR products were purified on a QIAquick PCR product purification spin column (QIAGEN, Hilden, Germany). For sequencing, we used sequencing primers (Table 2) in a commercially available sequencing kit (dRhodamine Terminator cycle Sequencing ready reaction with AmpliTaq Polymerase FS; PE Applied Biosystems, Warrington, United Kingdom) was used. For PCR products obtained using the fD1-357r primer pair, sequencing was performed using the primers fD1 and 357r. Sequencing products were purified by ethanol-magnesium precipitation, resolved on a 5% polyacrylamide gel (Long Ranger Singel packs, type 377-36cm WTR; Tebu, Le Perray en Yvelines, France) by electrophoresis with an ABI PRISM 377 DNA sequencer (Perkin-Elmer). For the 16S rRNA gene, partial sequences obtained were compared with that of *Escherichia coli* (43) to determine the position of base 170, which is the beginning of the 16S rRNA gene. For genes that encode for the 35-kDa protein and the Pap 31 protein of phage 60457, positions of signature sequences were determined according to the position of the open reading frame of the gene of *B. henselae* Houston-1 (GenBank accession numbers U21304 and AF001274, respectively). Thereafter, the gene sequences were compared for each strain.

Cell wall fatty acid analysis. Colonies of all the *B. henselae* strains described in this study were grown on Trypticase soy agar with 5% sheep blood (Becton Dickinson) at 37°C for 48 h and then saponified. Cell wall fatty acids were then extracted and analyzed by gas chromatography as previously reported (L. Miller and T. Berger, Hewlett-Packard company publication, 1985).

RESULTS

SDS-PAGE profiles of *B. henselae* strains. The Coomassie brilliant blue staining profiles of the eight strains of *B. henselae* examined by SDS-PAGE revealed dominant polypeptide bands of 60, 43, 29/30 (dual band at 29 and 30), and 32 kDa and a number of minor bands when solubilized at room temperature (Fig. 1). Two PAGE patterns could be determined based on the 29- and 30-kDa band (Fig. 1). PAGE pattern I, obtained for Houston-1, 90-615, and SA2 strains, showed a 29-kDa protein band, and PAGE pattern II, obtained for the URBHLLY8, URBHLIE9, Cat6, Fizz, and CAL-1 strains, consisted of a 30-kDa protein band (Fig. 1). The 32-kDa band was more intense in pattern I strains.

MIF reactivity and MAb isotype. The fusion of spleen cells from BALB/c mice immunized with *B. henselae* Houston-1

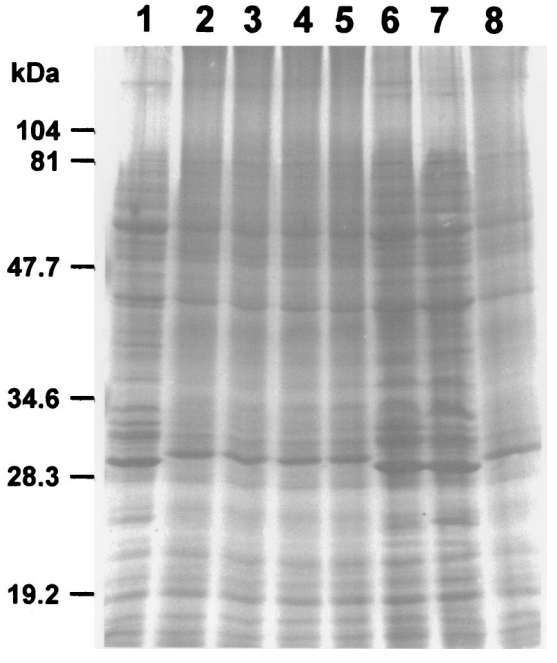


FIG. 1. Coomassie brilliant blue staining profiles of *B. henselae* strains following SDS-PAGE. Lanes: 1, Houston-1; 2, URBHLLY8; 3, URBHLIE9; 4, Cat6; 5, Fizz; 6, 90-615; 7, SA2; 8, CAL-1.

TABLE 3. Reciprocal titers of MAbs to *B. henselae* Houston-1 against different isolates of *B. henselae* by MIF

MAb	Reciprocal titer of MAb to *B. henselae* isolate		
	Houston-1	90-615	SA2
Bh1E10	2,048	512	512
Bh1H8	1,024	512	512
Bh2F7	1,024	512	512
Bh3H3	8,192	2,048	2,048
Bh8D4	4,096	1,024	1,024
Bh10E5	1,024	512	512
Bh12B8	8,192	1,024	1,024
Bh15B10	2,048	1,024	1,024
Bh18C2	2,048	1,024	1,024

with myeloma cells enabled the selection of 9 MAbs of the IgG3 subclass which could be used to differentiate strains of *B. henselae* (Table 3). All reacted to *B. henselae* Houston-1, 90-615, and SA2, but none reacted against *B. henselae* URBHL LY8, URBHLIE9, Cat6, Fizz, and CAL-1 or with strains of other *Bartonella* species presented in Table 1. The titers of the MAbs against Houston-1 were higher than those against the 90-615 and SA2 strains (Table 3). The titers of the MAbs against strains 90-615 and SA2 were, however, identical. In an MIF assay using Bh1H8 and the 22 *B. henselae* strains isolated from cats, 15 gave a positive reaction. Despite repeated attempts, we never obtained MAbs specific for strains URBHL LY8, URBHLIE9, Cat6, Fizz, and CAL-1.

Western immunoblotting. The nine MAbs reacted with the 29-kDa protein of the Houston-1, 90-615, and SA2 *B. henselae* strains but not with those of other *Bartonella* species tested. A weak reactivity was also observed with the 32-kDa protein of 90-615 and SA2 (Fig. 2). In Western blots where the antigens had been heated to 100°C for 5 min or digested in proteinase K, no MAb reactivity could be detected.

DNA-DNA relatedness. Relative reassociation with labeled DNA from strain URBHLLY8 was as follows: URBHLLY8, 100%; URBHLIE9, 92%; Fizz, 98%; CAL-1, 100%; Houston-1^T, 94%; 90-615, 89%; SA2, 98%.

Sequence analysis of amplified genes. The genotypes of our *B. henselae* strains were first defined according to the sequences of a small fragment of the 5′ end of the gene coding for 16S rRNA (Fig. 3). The beginning of the gene (base 170) was determined for each strain by comparing the sequence we obtained with that previously reported for *E. coli* in which the sequence of the gene is clearly defined. For genes that encode for the 35-kDa protein and the Pap 31 protein of phage 60457, positions of signature sequences were determined according to the position of the open reading frame. The sequences obtained for Houston-1, 90-615, and SA2 strains (genotype I in Fig. 3) were identical to one another but different from those of the URBHLLY8, URBHLIE9, Cat6, Fizz, and CAL-1 strains (genotype II in Fig. 3). ITS sequences revealed DNA similarity values from 91.3 to 99.7%, and each isolate had a different sequence. The PCR assays that allow amplification of a short fragment that contains signature sequences from genes encoding 16S rRNA, 35-kDa protein, Pap 31 protein allowed us to determine, after sequencing, that among the 22 *B. henselae* strains isolated from bacteremic cats, 15 belong to genotype I and 7 belong to genotype II. The 15 strains classified in genotype I were the 15 that gave positive reactions in the MIF assay using the Bh1H8 MAb.

Cell wall fatty acid analysis. All the *B. henselae* isolates were indistinguishable by using cell wall fatty acid analysis (data not shown).

DISCUSSION

The existence of genotypic variation among *B. henselae* strains has previously been demonstrated using different DNA-based techniques (2, 26, 28, 29, 35, 37, 38, 47). There is little information, however, on the antigenic variation between *B. henselae* strains, which might be important in the development

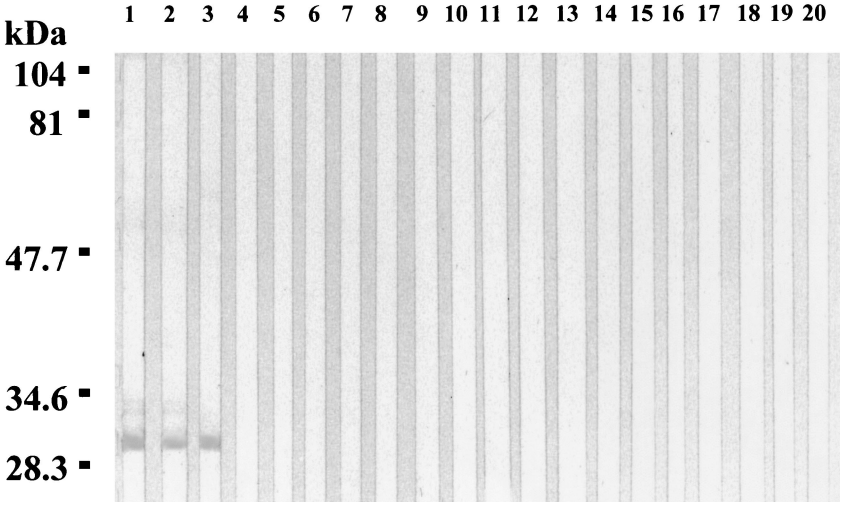
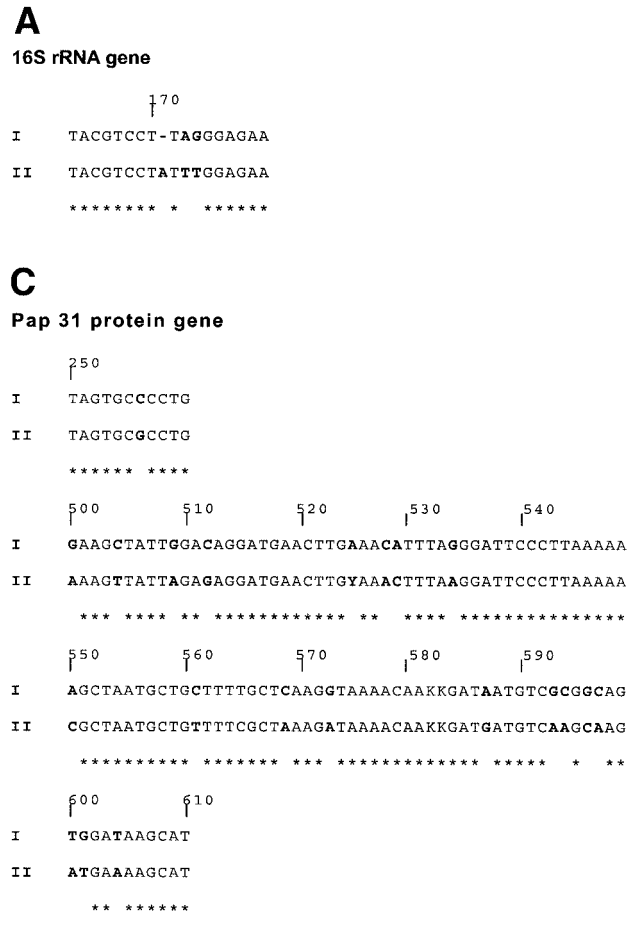


FIG. 2. Western immunoblotting of MAb Bh1E10 with *Bartonella* antigens. Lanes: 1, *B. henselae* 90-615; 2, *B. henselae* SA2; 3, *B. henselae* Houston-1; 4, *B. henselae* URBHLLY8; 5, *B. henselae* URBHLIE9; 6, *B. henselae* Cat6; 7, *B. henselae* Fizz; 8, *B. henselae* CAL-1; 9, *B. quintana* URBQLY4; 10, *B. quintana* Oklahoma; 11, *B. quintana* URBQPIEH2; 12, *B. quintana* Fuller 13, *B. elizabethae* F9251; 14, *B. grahamii* V2; 15, *B. taylorii* M6; 16, *B. doshiae* R18; 17, *B. vinsonii* Baker; 18, *Bartonella* sp. strain C1-phy; 19, *Bartonella* sp. strain C5-rat; 20, *B. bacilliformis* Monzon 812.



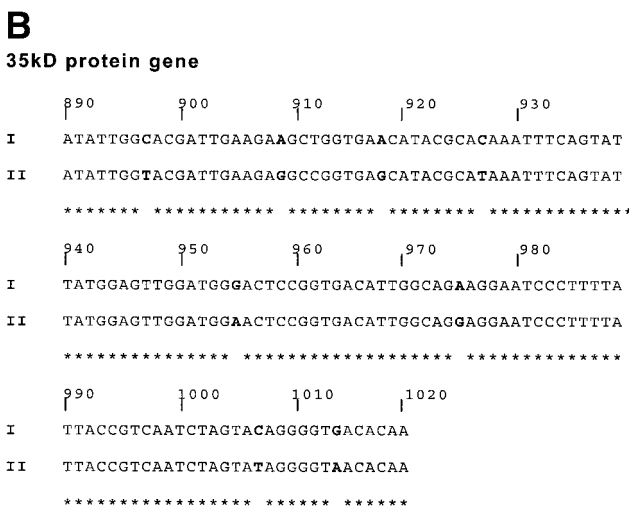


FIG. 3. Comparison of partial sequences of the 16S rRNA gene (A), the 35-kDa protein gene (B), and the Pap 31 protein (C) of Houston-1, 90-615, and SA2 strains (genotype I) and URBHLLY8, URBHLIE9, Cat6, Fizz, and CAL-1 strains (genotype II).

of improved diagnostic tests for *B. henselae* infections, the development of effective vaccines, and the discovery of new animal hosts and vectors. We tested eight strains of *B. henselae* from various places and from different hosts and clinical situations in order to evaluate whether the heterogeneity of the species was clustered into subspecies. The reactivity patterns of the MAbs we produced indicated that the *B. henselae* strains we had studied could be separated into two distinct groups, based on their antigenic properties, which is consistent with findings we reported previously (10). The first group, the Houston serotype, consisted of the Houston-1, 90-615, and SA2 strains, which could be detected by all the MAbs we produced. The second group, the Marseille serotype, against which none of the MAbs reacted, consisted of the URBHLLY 8, URBHLIE9, CAL-1, Fizz, and Cat6 strains. Our findings that the titers of the MAbs to the Houston-1 strain (against which they were produced) were consistently higher than those against the 90-615 and SA2 strains indicate that there are different levels of epitope expression among strains within the serotypes we propose. Further evidence for a distinct phenotypic difference between the two groups was provided by the results of SDS-PAGE study. The 29-kDa protein demonstrated in the Houston serotype strains was not present in the serotype Marseille and was replaced by a 30-kDa protein band (25). The 29-kDa protein was the antigen recognized by the Houston serotype-specific MAbs. Moreover, this heterogeneity can result in false-negative serology in patients infected with *B. henselae* serotype Marseille, for which antibody reactivity is tested against *B. henselae* serotype Houston, as previously reported (J. L. Mainardi, C. Figliolini, F. W. Goldstein, P. Blanche, M. Baret-Rigoulet, N. Galezowski, P. E. Fournier, and D. Raoult, Letter, J. Clin. Microbiol. **36:**2800, 1998). Curiously, in spite of repeated attempts of inoculation of strain URBHLLY8 to mice, we never succeeded in obtaining a MAb(s) directed only against the group including URBHLL Y8, URBHLIE9, CAL-1, Fizz, and Cat6 strains (unpublished data), indicating that the 29-kDa protein could be a modification of the 30-kDa protein in some strains.

This phenotypic clustering was consistent with genotypes determined by 16S rRNA type-specific PCR in The Netherlands (2) and Germany (37, 38). The strains we identified as belonging to genotype I were the same as those identified as belonging to the Houston serotype, based on the phenotypic data we obtained. Similarly, the strains in genotype II were the same as those belonging to the Marseille serotype. Moreover, these two serotypes have been previously shown to be two different pathotypes in cats, as they were unable to provide cross-protection (46). In this study, on the basis of consistent differences in three tested genes (the 16S rRNA gene, the 35-kDa protein gene, and the Pap 31 protein gene), for which the same variations were described in all tested strains, we confirmed that indeed two distinct genotypes exist. However, some genes, such as the ITS gene, showed a wide heterogeneity within each group. Moreover, DNA-DNA relatedness study did not divide the strains into the two clusters observed by serotyping with MAb or sequencing of selected genes. The division into two clusters seems to be based on a limited polymorphism that affects only a limited part of the genome that codes for the major protein epitope recognized by our MAbs and the 35-kDa and Pap 31 proteins. The future publication of the complete genome of the *B. henselae* Houston-1^T strain, which is now undergoing sequencing (http://www.ncbi.nlm.nih

.gov/PMGifs/Genomes/bact.html), may help to better the understanding of epitopes involved in reactions with our MAbs. Nevertheless, our results present both phenotypic and genotypic evidence that there are two distinct groups within the *B. henselae* species. The existence of these two groups may be responsible for possible reinfection in cats, such as that described by Yamamoto et al., who demonstrated a lack of heterologous protection among each of two genotypes or serotypes of *B. henselae* (46).

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