

Species-Specific Monoclonal Antibodies for Rapid Identification of *Bartonella quintana*

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Seven species-specific monoclonal antibodies (MAbs) to *Bartonella quintana* were produced and characterized. The MAbs were of the immunoglobulin G class and reacted only with 13 *B. quintana* strains in indirect microimmunofluorescence and Western immunoblotting assays. They did not react with eight other *Bartonella* spp., including *Bartonella henselae*, the most closely related species, and a selected MAb did also not react with nine other strains of gram-negative bacteria. The MAbs reacted mainly with a 34-kDa protein epitope of *B. quintana* which was shown to be species specific by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Four of five body lice experimentally infected with *B. quintana* were found to be positive for the organism in microimmunofluorescence assays with one MAb. These MAbs may provide a specific, simple, rapid, and low-cost tool for the identification of *B. quintana* and the diagnosis of infections due to the microorganism.

Bartonella spp. are gram-negative, short-rod bacteria. Presently there are 14 recognized species within the genus *Bartonella* (2, 3, 9, 11, 12), and of these, four species are currently recognized as human pathogens: *B. bacilliformis*, *B. quintana*, *B. henselae*, and *B. elizabethae* (6). *B. bacilliformis* was the earliest species of this genus to be described (23) and is the agent of Carrion's disease. Infections with *B. bacilliformis* have yet to be reported from outside a very restricted geographic region in the Andes of western South America. *B. quintana* was first recognized during World War I as the etiological agent of trench fever. Although Vinson and Fuller (28) isolated the organism in 1961, there was little scientific interest in the organism or trench fever for the next 20 years, as they were apparently only very rarely encountered. Recent investigations, however, have led to the reemergence of *B. quintana* as an organism of medical importance. Bacillary angiomatosis was initially characterized by the appearance of multiple cutaneous lesions, which were assumed to be infectious because these lesions contained bacilli that stained with Warthin-Starry stain (1, 5, 16) and resolved with antibiotic treatment (5). Subsequently the observed bacillus was characterized by PCR and 16S rRNA gene sequencing, which showed it to be a new organism closely related to *B. quintana* (22), and in 1992 *B. quintana* was isolated from skin lesions of bacillary angiomatosis patients (14). The organism has also been found to be associated with other, less specific clinical syndromes, such as bacteremia (26), endocarditis (7, 19, 27), chronic lymphadenopathy (20), neurological disorders (29), and chronic bacteremia in homeless patients (4). There is a need, then, for rapid and specific methods to identify *B. quintana* and differentiate it from other *Bartonella* species. In this report we describe the characteristics and specificities of seven species-specific monoclonal antibodies (MAbs) that we produced against *B. quintana*.

MATERIAL AND METHODS

***Bartonella* strains.** The sources of the *Bartonella* strains used in the study are listed in Table 1. *Bartonella* isolates were grown on Columbia blood agar con-

taining 5% whole sheep blood (BioMerieux, Marcy l'Etoile, France) at 37°C with a 5% carbon dioxide atmosphere, except for *B. bacilliformis*, which was grown at 32°C. After 5 to 7 days of culture, the organisms were harvested and suspended in sterile deionized water for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or with phosphate-buffered saline (pH 6.8) (PBS) for microimmunofluorescence (MIF).

Production of MAbs. For production of MAbs (10), 6-week-old female BALB/c mice were inoculated three times intraperitoneally with 2×10^4 *B. quintana* Fuller organisms, suspended in 0.5 ml of PBS, at 7-day intervals. One week after the final intraperitoneal inoculation the mice were injected intravenously with 4×10^3 organisms suspension in 0.1 ml of PBS. Three days later, spleen cells from the mice were fused with SP2/0-Ag14 myeloma cells (10:1) by 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 17% fetal bovine serum (Gibco BRL) and hypoxanthine-aminopterin-thymidine selective medium (Sigma Chemical Co.) at 37°C in a humidified atmosphere supplemented with 5% CO₂. The supernatants were screened for antibodies to *B. quintana* by MIF, and positive 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 Chemical Co.). Specificities of MAbs were tested by Western immunoblotting.

MIF assay. The MIF assay (18) was used to screen hybridoma clones and to determine the specificities of MAbs. Antigens were placed on 24-well microscope slides with a pen nib. The antigens were fixed in methanol for 10 min at room temperature and incubated in a humidified chamber at 37°C for 30 min. After two washes in PBS (5 min each) and rinsing in sterile distilled water, the slides were air dried at 37°C. Following incubation at 37°C for 30 min with dechlorotriazinyl amino fluorescein-conjugated goat anti-mouse IgG plus IgM (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) diluted 1:200 in PBS with 0.2% Evans blue (BioMerieux), the slides were washed as described above and mounted with Fluoprep (BioMerieux) before being read under an epifluorescence microscope (Axioskop20; Carl Zeiss, Gottingen, Germany) at a magnification of $\times 400$. Sera from immunized mice were used as positive controls, and sera from healthy unexposed mice were used as negative controls.

SDS-PAGE and Western immunoblotting. Antigens were suspended in an equal volume of sample buffer (0.0625 M Tris hydrochloride [pH 8.0], 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.02% bromophenol blue) (15) and separated electrophoretically in 12% resolving gels with 5% stacking gels at a constant current of 8 to 10 mA per gel for 3 to 4 h in running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS) in a Mini Protein II apparatus (Bio-Rad, Richmond, Calif.). Prestained SDS-PAGE standards (low range; Bio-Rad) were used as a reference. The separated antigens were transferred to 0.45- μ m-pore-size nitrocellulose membranes (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). After transfer, the nitrocellulose membranes were incubated overnight in PBS with 5% nonfat dry milk to block nonspecific binding sites. After three 10-min washes in PBS, the membranes were air dried and cut into strips, which were incubated with MAbs diluted 1:10 in PBS containing 3% nonfat dry milk at room temperature for 1 h and washed as described above. After incubation at room temperature for 1 h with peroxidase-conjugated F(ab')₂ fragment goat anti-mouse IgG (heavy and light chains) (AffiniPure; Jackson ImmunoResearch) diluted 1:200 in PBS with 3% nonfat dry milk and three washes in PBS,

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TABLE 1. Strains of *Bartonella* used for screening and determination of specificity of MABs

Species	Strain	Source ^a
<i>B. bacilliformis</i>	Monzon 812 Monzon 269	
<i>B. henselae</i>	Houston-1 ^T URBHLLY8 URBHLLIE9	ATCC 49882 CSD; Marseille, France IE; Marseille, France
<i>B. quintana</i>	Fuller ^T URBQMLY4 Oklahoma URBQPIEH2	ATCC VR-358 LY; Marseille, France Chronic adenopathy; Oklahoma City, Okla. IE; Marseille, France
<i>B. elizabethae</i>	F9251 ^T	ATCC 49927
<i>B. grahamii</i>	V2 ^T	NCTC 12860
<i>B. taylorii</i>	M6 ^T	NCTC 12861
<i>B. doshiae</i>	R18 ^T	NCTC 12862
<i>B. vinsonii</i>	Baker ^T	ATCC VR-152
<i>B. henselae</i>	Cat 6 Fizz King SA2 CAL-1	South Africa South Africa United States United States United States
<i>B. quintana</i>	SH-perm URBQTBAAH1 URBQMLY15 URBQPBA7 URBQMLIEH6 URBQMTF14 URBQMTF15 URBQMTF17 URBQMTF12	Russia BA; Marseille, France LY; Marseille, France BA; Marseille, France IE; Marseille, France TF; Marseille, France TF; Marseille, France TF; Marseille, France TF; Marseille, France TF; Marseille, France
<i>B. clarridgeiae</i>	URBCMHC26	Cat's blood, France

^a BA, bacillary angiomatosis; CSD, cat scratch disease; IE, infective endocarditis; LY, lymphadenopathy; TF, trench fever.

color was developed with coloring buffer containing 0.015% 4-chloro-1-naphthol and 0.015% hydrogen peroxide in 16.7% methanol in Tris-buffered saline.

Blind testing of bacteria with MABs. Blind testing of 26 bacteria by MIF with MAb Bq73H4 was carried out with 13 *B. quintana* strains isolated from patients in Marseille, France, by our laboratory; 5 *Bartonella* strains of other species; and *Escherichia coli*, *Desulfovibrio fairfieldensis*, *Enterococcus faecalis*, *Afpia cleavelandensis*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Afpia felis*, and *Klebsiella pneumoniae*.

Detection of *Bartonella* in lice preparations. Twenty milliliters of a suspension of 10¹⁰ *B. quintana* Oklahoma organisms per ml was injected intravenously into a New Zealand White rabbit over 30 min. Following the injection, five human body lice were applied to the previously shaved belly of the rabbit and allowed to feed for 15 min. After being crushed and smeared onto microscope slides, these body lice were tested for *B. quintana* by MIF with undiluted hybridoma Bq73H4 supernatant as described above.

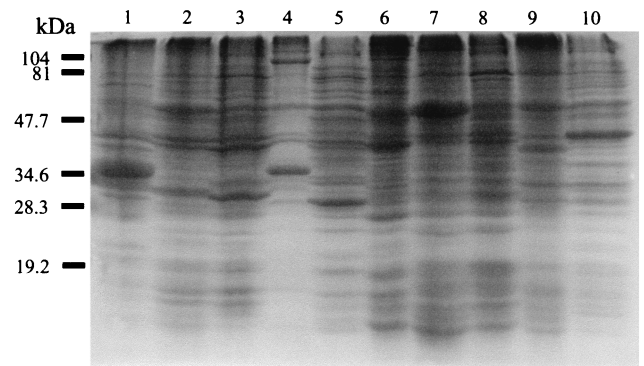


FIG. 1. Coomassie brilliant blue staining profiles of *Bartonella* strains on SDS-PAGE. Lanes: 1, *B. bacilliformis*; 2, *B. henselae* subspecies *houstoniae*; 3, *B. henselae* subspecies *massiliae*; 4, *B. clarridgeiae*; 5, *B. quintana*; 6, *B. elizabethae*; 7, *B. grahamii*; 8, *B. taylorii*; 9, *B. doshiae*; 10, *B. vinsonii*. Molecular mass markers were loaded on the left.

RESULTS

MIF reactivities and isotypes of MABs. Seven MABs of subclasses IgG3, IgG1, and IgG2a (Table 2) produced from subcloned hybridomas were examined for their reactivities with *Bartonella* strains. These MABs were reactive at identical titers with all *B. quintana* strains tested, except strain URBQPIEH2 (Table 2), and did not react at all with the other *Bartonella* species tested.

SDS-PAGE. Although the SDS-PAGE profiles of the different species of *Bartonella* differed (Fig. 1), a 34-kDa protein appeared to be specific for *B. quintana*, and this was also the most prominent band in the SDS-PAGE profiles.

Western immunoblotting. The seven MABs reacted with a 34-kDa epitope of all *B. quintana* strains but did not react with any antigens of the other *Bartonella* species (Fig. 2). In Western blots of *B. quintana* antigens that had been digested with proteinase K, the MABs failed to react with the 34-kDa epitope. Heating of the antigens (100°C for 10 min) before immunoblotting did not, however, affect the reactivities of the MABs (Fig. 3).

Blind testing of bacteria with MABs. The supernatant from hybridoma Bq73H4 reacted with all of the strains of *B. quintana* tested but did not react with the other *Bartonella* species or with the other bacteria used in the study.

Detection of *B. quintana* in lice. *Bartonella* could be demonstrated in four of the five infected lice by MIF with MABs Bq73H4 (Fig. 4), Bq85G7, and Bq100E9.

TABLE 2. Subclasses of MABs and titers to different *B. quintana* isolates

MAB	IgG subclass	Titer to <i>B. quintana</i> strain ^a :												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Bq73H4	1	1,280	1,280	1,280	640	1,280	1,280	1,280	1,280	1,280	1,280	1,280	1,280	1,280
Bq85G7	3	1,280	1,280	1,280	320	1,280	1,280	1,280	1,280	1,280	1,280	1,280	1,280	1,280
Bq86D2	3	320	320	320	40	320	320	320	320	320	320	320	320	320
Bq90H6	2a	320	320	320	160	320	320	320	320	320	320	320	320	320
Bq99C9	3	2,560	2,560	320	160	2,560	2,560	2,560	2,560	2,560	2,560	2,560	2,560	2,560
Bq100E9	3	2,560	2,560	640	160	2,560	2,560	2,560	2,560	2,560	2,560	2,560	2,560	2,560
Bq100G5	3	2,560	2,560	320	1,280	2,560	2,560	2,560	2,560	2,560	2,560	2,560	2,560	2,560

^a *B. quintana* strains: 1, Fuller; 2, URBQMLY4; 3, Oklahoma; 4, URBQPIEH2; 5, SH-perm; 6, URBQTBAAH1; 7, URBQMTF17; 8, URBQMTF12; 9, URBQMLY15; 10, URBQPBA7; 11, URBQMLIEH6; 12, URBQMTF14; 13, URBQMTF15.

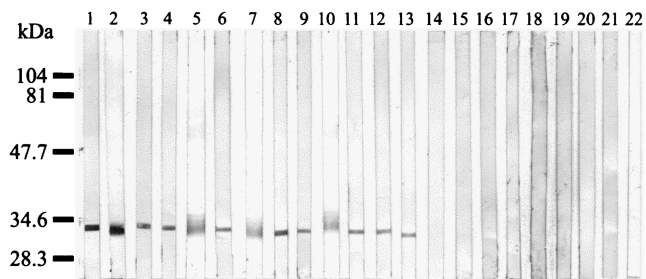


FIG. 2. Western immunoblotting of MAb Bq100G5 with *Bartonella* antigens. Lanes: 1, *B. quintana* Fuller; 2, *B. quintana* URBQMLY4; 3, *B. quintana* Oklahoma; 4, *B. quintana* URBOPIEH2; 5, *B. quintana* SH-perm; 6, *B. quintana* URBQTBAAH1; 7, *B. quintana* URBQMTF17; 8, *B. quintana* URBQMTF12; 9, *B. quintana* URBQMLY15; 10, *B. quintana* URBQPBA7; 11, *B. quintana* URBQLIEH6; 12, *B. quintana* URBQMTF14; 13, *B. quintana* URBQMTF15; 14, *B. bacilliformis* Monzon 812; 15, *B. henselae* Hounton-1; 16, *B. henselae* URBHLLY 8; 17, *B. elizabethae*; 18, *B. grahamii* V2; 19, *B. taylorii* M6; 20, *B. doszhae* R18; 21, *B. vinsonii* Baker; 22, *B. clarridgeiae*. Molecular mass markers were loaded on the left.

DISCUSSION

Several methods for confirming the identification of presumptive *Bartonella* spp. have been described, ranging from the comparison of the biochemical reactivities of isolates to more complex genotypic and phenotypic analyses. While *Bartonella* spp. were once considered to be relative inert, our laboratory (8) found that the introduction of 100 µg of hemin per ml to the test media resulted in the bacteria giving positive reactions in a number of biochemical tests. This then enabled the effective differentiation of *B. quintana*, *B. henselae*, and *B. vinsonii* from one another. The differentiation of *Bartonella* spp. has also been shown to be possible by using an extensive range of genotypic analyses, and DNA-DNA hybridization has clearly demonstrated the taxonomic position of *Bartonella* spp. (3, 6, 21). The 16S rRNA gene sequences of *Bartonella* spp. have been shown to be similar but unique for each species (3, 6, 17, 21), and *Bartonella* spp. can be differentiated by restriction fragment length polymorphism analyses of amplified 16S rRNA gene products with a combination of *DdeI* and *MnII* (2). Similarly, PCR-restriction fragment length polymorphism analysis of the citrate synthase gene enables the identification of *Bartonella* spp. (13, 17), which can also be differentiated by pulsed-field gel electrophoresis with various restriction enzymes (17).

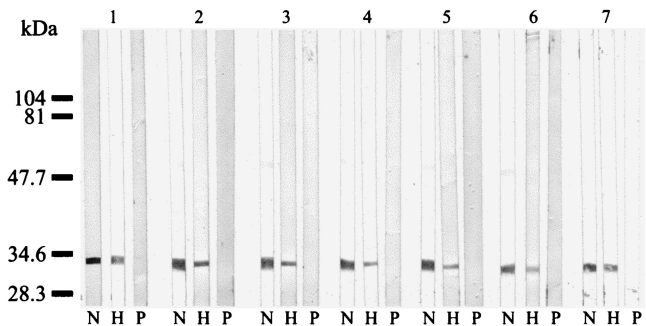


FIG. 3. Immunoblotting of *B. quintana* Fuller antigens treated with MAb in different ways. Lanes N, native antigens without treatment; lanes P, antigens treated with proteinase K (Boehringer, Mannheim, Germany) at 1.5 mg/ml at 37°C for 1.5 h; lanes H, antigens heated at 100°C for 5 min. MAbs: group 1, Bq73H4; group 2, Bq85G7; group 3, Bq86D12; group 4, Bq90H6; group 5, Bq99C9; group 6, Bq100E9; group 7, Bq100G5. Molecular mass markers were loaded on the left.

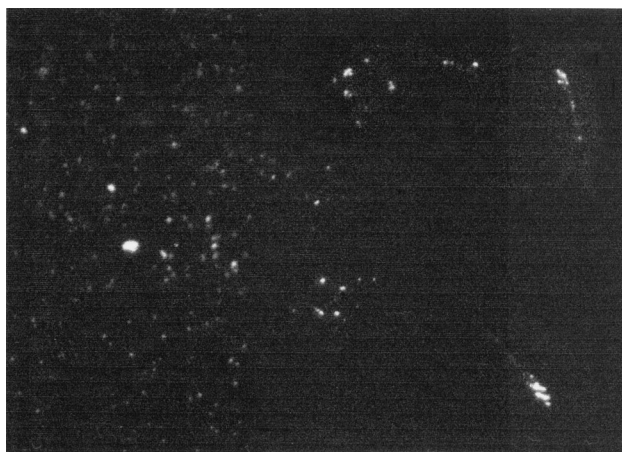


FIG. 4. Fluorescent antibody-stained organisms of *B. quintana* on a crushed infected-lice smear with supernatant of undiluted hybridoma Bq73H4.

Only a few phenotypic characteristics have been reported for the identification of *Bartonella* at the species level (17, 25). In our experiments, we were able to produce and characterize species-specific MAbs to *B. quintana*. All seven of our MAbs reacted with various antigens of *B. quintana* in MIF and Western immunoblotting assays. The MAb (Bq73H4) selected for diagnostic purpose did not react with antigens of other *Bartonella* species as determined with eight strains of other bacteria, which clearly indicates that it is species specific for *B. quintana*. We note that the reactivity of our MAbs with *B. quintana* isolate URBOPIEH2 was relatively weak. This indicates that there might be antigenic differences between strains of *B. quintana*, and further research to resolve this issue is under way in our laboratory.

The MAbs that we describe in this report appear, then, to be suitable for use in rapid, simple, accurate, and low-cost tests such as direct and indirect MIF assays to differentiate *Bartonella* spp. The use of the MAbs would appear to be particularly useful in differentiating *B. quintana* and *B. henselae*, organisms which cause similar clinical signs in patients and have to date been differentiated only by using more expensive and complicated genotypic analyses. The MAbs could also be of use in epidemiological studies, as we were able to use MAbs Bq73H4, Bq85G7, and Bq100E9 to detect *B. quintana* by MIF in four of the five lice that we experimentally infected with the organism, and could be complementary with PCR detection (24).

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