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Production of *Bartonella* Genus-Specific Monoclonal Antibodies

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Monoclonal antibodies (MAbs) which react with heat-resistant proteins with molecular masses of 32 to 33 kDa of 14 different *Bartonella* species were produced. These antibodies did not react with antigens of 26 diverse bacterial strains by microimmunofluorescence assay except MAb B3D4, which reacted with *Chlamydia psittaci* and *Chlamydia trachomatis* at low titers. The identification of a common *Bartonella* antigenic protein will make it possible to later produce a diagnostic antigen by cloning and expressing it in *Escherichia coli*. Moreover, these MAbs allow all *Bartonella* species to be identified to the genus level.

The genus *Bartonella* currently comprises 14 species. Human infections due to *Bartonella* species are widely considered emerging diseases, although they also include long-recognized diseases such as Carrión's disease, trench fever, and cat scratch disease (15, 17, 23). Newer clinical manifestations, such as bacillary angiomatosis, peliosis hepatis, chronic lymphadenopathy, and endocarditis, which are sometimes due to uncommonly encountered species such as *Bartonella elizabethae, Bartonella vinsonii* subsp. *berkhoffii*, or *Bartonella vinsonii* subsp. *arupensis*, have been recently identified (1, 23, 25, 28, 32). Serologic diagnosis of *Bartonella* spp. is mostly based on microimmunofluorescence (MIF) serology that detects antibodies to *B. quintana* and *B. henselae* only (21, 23). A serologic test that detects antibodies against all species is not available. Such a test needs to detect an epitope common to, but also specific to, all *Bartonella* spp. A monoclonal antibody (MAb) that can recognize this epitope would be the first step towards detecting this antigen after cloning and expressing the *Bartonella* genome in *Escherichia coli* in order to produce it for use in an enzyme-linked immunosorbent assay. *Bartonella* spp. may be isolated from clinical samples by using cell culture systems with endothelial cells or blood- or hemin-containing axenic media (21, 29). When isolated, identification of *Bartonella* is mostly based on molecular methods. The availability of a MAb that could screen *Bartonella* at the genus level would avoid the use of expensive and time-consuming molecular procedures on non-*Bartonella* bacteria. We thus decided to produce and characterize *Bartonella* genus-specific MAbs.

The sources of *Bartonella* strains used to screen hybridomas and test the specificity of MAbs are presented in Table 1. *Bartonella* strains were harvested and suspended in deionized water for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or in phosphate-buffered saline (PBS) for the MIF assay after 5 to 7 days of culture on blood agar plates. The procedure for the production of MAbs has been detailed elsewhere (12, 22). Briefly, 6-week-old female BALB/c mice were inoculated with *B. henselae* Houston-1 suspended in 0.5 ml of PBS. The supernatants of the hybridomas were screened for antibodies to *B. henselae* by MIF. Representative hybridomas were subcloned twice by limiting dilution. Isotypes of MAbs were determined with an Immuno Type mouse monoclonal antibody isotyping kit with antisera to mouse immunoglobulin M (IgM), IgA, IgG1, IgG2a, IgG2b, and IgG3 (Sigma). Ascitic fluids were produced by injecting about 3×10^6 cells of hybridoma (B2D3 and B3D4) suspended in 0.5 ml of PBS into the mice 1 week after an intraperitoneal injection of 0.5 ml of pristane (2,6,10,14-tetramethylpentadecane; Sigma). The MIF assay (26) was used to screen hybridoma clones and to determine the specificity of the MAbs. Blind testing of 45 bacteria by MIF with MAbs B2D3 and B3D4 was carried out on 19 *Bartonella* strains, 3 *Chlamydia* strains, and 23 bacterial strains isolated in our laboratory from clinical samples (Table 1). Sera from immunized mice were used as positive controls, and sera from healthy mice were used as negative controls. SDS-PAGE and Western blotting were performed according to a modification of the method described by Laemmli (19, 22). Five human body lice from a laboratory colony were infected with a *B. quintana* strain by feeding on a bacteremic rabbit previously infected intravenously by 10^8 *B. quintana* cells. *B. quintana* bacteremia at the time the lice were fed was assessed by blood culture as previously described for cats (3). After being crushed and smeared onto microscope slides the lice were tested for *Bartonella* by MIF as described above with ascitic fluid of hybridoma B2D3 diluted 1:1,000.

SDS-PAGE analysis of *Bartonella* antigens demonstrated distinct profiles of *Bartonella* species. Depending on species, 12 to 35 bands were observed. Proteins of 85, 71, 54, 44 to 47, 40, 36, 32 to 33, 30, and 18 to 19 kDa were common to all *Bartonella* strains studied (Fig. 1a). Both MAbs reacted with all tested *Bartonella* species. The immunofluorescence assay titers of MAbs with different *Bartonella* bacteria showed obvious differences. Titers from the homologous strain Houston-1 were the highest. The isotypes of B2D3 and B3D4 were identified as subclass IgG1. MAbs B2D3 and B3D4 showed reactivity with 32- or 33-kDa protein bands (Fig. 1b). The MAbs were di-

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TABLE 1. Reactivity of MAbs with *Bartonella* antigens

Species	Strain	Source[a]	Titer of MAb:	
			B2D3	B3D4
B. henselae	Houston-1 (ATCC 4988)	Bacteremia (27)	6,400	12,800
B. henselae	San Ant 2 (SA2)	Cat scratch disease (6)	25,600	25,600
B. henselae	CAL-1	Septicemia, United States	6,400	3,200
B. henselae	URBHLLY8 (CIP 104756)	Cat scratch disease (7)	6,400	3,200
B. henselae	URBHLIE9	Endocarditis (7)	6,400	6,400
B. quintana	URBQMLY15	Chronic lymphadenopathy (9, 21)	6,400	12,800
B. quintana	Fuller (ATCC VR-358)	Trench fever (30)	6,400	12,800
B. quintana	SH-PERM	Trench fever, Russia	6,400	12,800
B. clarridgeiae	URBCMNHC26	Blood of cat, France	6,400	12,800
B. elizabethae	F9251 (ATCC 49927)	Endocarditis (5)	6,400	3,200
B. grahamii	V2 (NTCC 12860)	Blood of *Clethrionomys glareolus* (2)	6,400	3,200
B. taylorii	M6 (NTCC 12861)	Blood of *Apodemus* spp. (2)	3,200	6,400
B. doshiae	R18 (NTCC 12862)	Blood of *Microtus agrestis* (2)	6,400	12,800
B. vinsonii subsp. *vinsonii*	Baker (ATCC VR-152)	Spleen of *Microtus pennsylvanicus* (31)	3,200	3,200
B. vinsonii subsp. *arupensis*	OK 94-513 (ATCC 700727)	Bacteremia (32)	6,400	12,800
B. vinsonii subsp. *berkhoffii*	NCSV93-CO1 (ATCC 51672)	Blood of dog (18)	1,600	3,200
B. alsatica	IBS 383 (CIP 105477)	Blood of rabbit (13)	6,400	12,800
B. koehlerae	C-29 (ATCC 700693)	Blood of cat (10)	6,400	12,800
B. tribocorum	IBS 506 (CIP 105476)	Blood of rat (14)	1,600	6,400
B. bacilliformis	Monzon 812	Blood of bartonellosis patient, Peru	3,200	12,800
C. psittaci			50	800
C. trachomatis			<25	400
C. pneumoniae			<25	<25
23 species[b]			<25	<25

[a] Geographic origin is given if the isolation of the strain is not detailed elsewhere.
[b] Includes *E. coli, Klebsiella pneumoniae, Enterobacter aerogenes, Yersinia enterocolitica, Shigella dysenteriae, Salmonella enterica, Campylobacter jejuni, Brucella melitensis, Ochrobactrum anthropi, Haemophilus influenzae, Kingella kingae, Neisseria meningitidis, Bacteroides fragilis, Desulfovibrio fairfieldensis, Fusobacterium necrophorum, Enterococcus faecalis, Afipia clevelandensis, Afipia felis, Pseudomonas aeruginosa, Pseudomonas putida, Burkholderia cepacia, Stenotrophomonas maltophilia,* and *Coxiella burnetii*.

rected against heat-resistant proteins because digestion with proteinase K completely destroyed the antigen's reactivities and heat treatment at 100°C for 10 min did not. The ascitic fluid from hybridomas B2D3 and B3D4 reacted with all of the *Bartonella* strains tested, but it did not react with the 23 other bacteria tested. Cross-reactivity was observed with *Chlamydia psittaci* and *Chlamydia trachomatis*. Nevertheless, the immunofluorescence assay titers of MAbs to *Chlamydia* spp. were much lower (Table 1). *Bartonella* spp. were demonstrated in four of the five infected lice by MIF with MAbs B2D3 and B3D4.

The clinical manifestations of infections due to *Bartonella, Coxiella*, and *Chlamydia* can often be confused, especially in cases of infectious endocarditis. However, differential diagnosis of the diseases is important because their treatments are different. As *Chlamydia* spp. and *Coxiella burnetii* are strictly intracellular bacteria and *Bartonella* spp. are fastidious slowly growing organisms, they are difficult to isolate. Therefore, diagnosis of these infections continues to rely mainly on serology in spite of the serological cross-reactions among members of these genera that have been described (11, 20, 24). Moreover, because recently described species such as *B. elizabethae, B.*

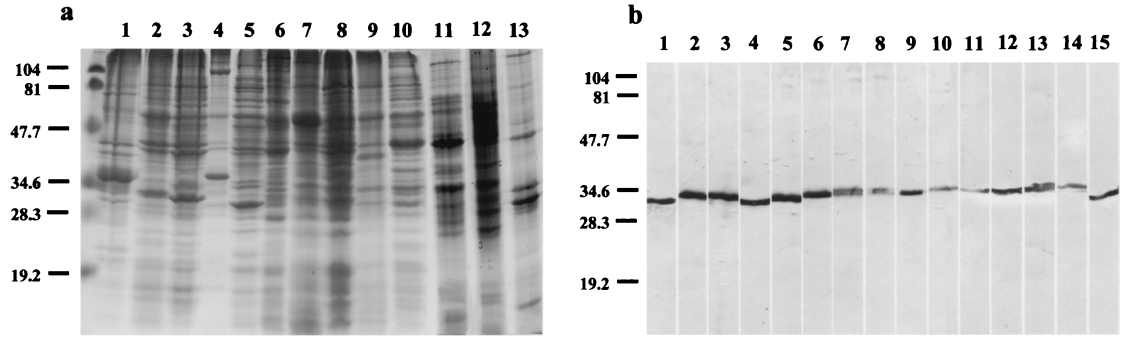

FIG. 1. (a) SDS-PAGE analysis of different *Bartonella* species. Lanes: 1, *B. bacilliformis*; 2, *B. henselae* Houston-1; 3, *B. henselae* URBHLLY8; 4, *B. clarridgeiae*; 5, *B. quintana*; 6, *B. elizabethae*; 7, *B. grahamii*; 8, *B. taylorii*; 9, *B. doshiae*; 10, *B. vinsonii* subsp. *vinsonii*; 11, *B. tribocorum*; 12, *B. koehlerae*; 13, *B. alsatica*. (b) Western immunoblotting of MAb B2D3 with *Bartonella* antigens. Lanes: 1, *B. bacilliformis*; 2, *B. henselae* Houston-1; 3, *B. henselae* URBHLLY8; 4, *B. clarridgeiae*; 5, *B. quintana*; 6, *B. elizabethae*; 7, *B. grahamii*; 8, *B. taylorii*; 9, *B. doshiae*; 10, *B. vinsonii* subsp. *vinsonii*; 11, *B. vinsonii* subsp. *berkhoffii*; 12, *B. vinsonii* subsp. *arupensis*; 13, *B. tribocorum*; 14, *B. koehlerae*; 15, *B. alsatica*. Molecular masses (in kilodaltons) are shown at left.

vinsonii subsp. *berkhoffii, B. vinsonii* subsp. *arupensis*, and *B. clarridgeiae* may be encountered in humans, a specific serologic test that recognizes all *Bartonella* infections is needed. In our work, we have obtained, as a first step, MAbs that recognize a protein antigen common to all *Bartonella* species. After cloning the *Bartonella* sp. genome in *E. coli* in order to obtain an expression bank, these MAbs could be used for screening products of clones in order to obtain a protein antigen common to all *Bartonella* spp. that could be used in an enzyme-linked immunosorbent assay for the detection of antibodies to all *Bartonella* spp. The anti-*Bartonella* genus-specific MAbs obtained in this study are highly specific, as they did not cross-react with 26 other bacterial species, except that MAb B3D4 cross-reacted at low titers with *C. trachomatis* and *C. psittaci*. Interestingly, none of the MAbs obtained reacted with *Chlamydia pneumoniae* or *Coxiella burnetii*, in spite of the cross-reactivity of these two genera and *Bartonella* spp. which has been previously described (11, 20, 24). Serological cross-reactivity between *B. bacilliformis* and *C. psittaci* antigens has been demonstrated previously (16). It was associated with a cross-reacting lipopolysaccharide antigen. Cross-reactivity between *B. quintana, C. psittaci*, and *C. pneumoniae* was later demonstrated in patients with *B. quintana* endocarditis (8). The serological cross-reactivity of *Bartonella* sp. and *Chlamydia* sp. antigens in the sera of patients infected by a member of these genera was also demonstrated to be due to cross-reacting protein antigens with molecular masses ranging from 30 to 90 kDa (24). Thus, our MAb B3D4 could also be used to investigate cross-reacting epitopes between *Bartonella* spp. and *Chlamydia* spp.

In conclusion, our *Bartonella* genus-specific MAbs recognized specifically all the tested species of *Bartonella*, and they successfully detected *B. quintana* in body lice. Thus, our MAbs may provide a tool to identify, at the genus level, isolated bacteria for which presumptive identification is compatible with *Bartonella* spp. or to detect such bacteria within arthropods, avoiding the use of molecular techniques for screening (4, 21).

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