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 Carrion’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 henselae*, *Bartonella quintana*, *Bartonella burgdoferi*, and *Bartonella clarridgeiae*, 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 all *Bartonella* species to the genus level. MAbs allow all *Bartonella* species to be identified to the genus level.

The sources of *Bartonella* strains used to screen hybridomas and test the specificity of MAbs are presented in 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). 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 × 10⁶ 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 10⁸ *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-
rected against heat-resistant proteins because digestion with proteinase K completely destroyed the antigen’s reactivities because recently described species such as these genera that have been described (11, 20, 24). Moreover, 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 burnetti 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.

### TABLE 1. Reactivity of MAbs with Bartonella antigens

| Species                  | Strain                          | Source[a]  | Titer of MAb: |
|--------------------------|---------------------------------|------------|---------------|
| 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              | URBHLLIE9                       | Endocarditis (7) | 6,400 6,400 |
| B. quintana              | URBOMLY15                       | 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. claridgeiae           | URBCMNHIC26                     | Blood of cat, France | 6,400 12,800 |
| B. elizabethae           | F9251 (ATCC 49927)              | Endocarditis (5) | 6,400 3,200 |
| B. grahamii              | V2 (NTCC 12660)                 | Blood of Clostridium difficile (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 pennsylvania (31) | 3,200 3,200 |
| B. vinsonii subsp. arupensis | OK 94-513 (ATCC 700727)     | Bacteremia (32) | 6,400 12,800 |
| B. vinsonii subsp. berkhoftii | 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 70693)               | 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. psitaci               |                                 |             | 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, Königella kingae, Nisseria meningitidis, Bacteroides fragilis, Desulfovibrio fairfieldensis, Fusobacterium necrophorum, Entersoccus faecalis, Afipia clevelandensis, Afipia felis, Pseudomonas aeruginosa, Pseudomonas putida, Burkholderia cepacia, Stenotrophomonas maltophilia, and Coxiella burnetti.

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. claridgeiae; 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. claridgeiae; 5, B. quintana; 6, B. elizabethae; 7, B. grahamii; 8, B. taylorii; 9, B. doshiae; 10, B. vinsonii subsp. vinsonii; 11, B. vinsonii subsp. berkhoftii; 12, B. vinsonii subsp. arupensis; 13, B. tribocorum; 14, B. koehlerae; 15, B. alsatica. Molecular masses (in kilodaltons) are shown at left.
Bartonella clarridgeiae may be encountered in humans, a specific serologic protein antigen common to all Bartonella spp. 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 reacted specifically with all the tested species of Bartonella, Chlamydia, and Rochalimaea spp. Interestingly, none of the MAbs obtained reacted with Bartonella henselae, Bartonella quintana, and Afipia felis isolated from 64 patients with suspected cat-scratch disease, Scand. Infect. Dis. 28:361–366.

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