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

ZHONGXING LIANG AND DIDIER RAOULT*

Unité des Rickettsies, CNRS UPRES-A 6020, Faculté de Médecine, Université de la Méditerranée, Marseille, France

Received 27 May 1999/Returned for modification 20 July 1999/Accepted 27 September 1999

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 Carrión’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-
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. The supernatant from hybridoma Bq73H4 reacted with a 34-kDa protein antigen 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 SDS-PAGE did not, however, affect the reactivities of the MAbs (Fig. 3).

**Detection of Bartonella in lice preparations.** Twenty milliliters of a suspension of 10^8 B. quintana 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 MIF with undiluted hybridoma Bq73H4 supernatant as described above.

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

| MAb     | IgG subclass | Titer to B. quintana strain* |
|---------|--------------|------------------------------|
|         |              | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  |
| Bq73H4  | 1            | 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280|
| Bq5G7   | 3            | 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280| 1,280|
| Bq5D2   | 2            | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  |
| Bq9H6   | 2a           | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  | 320  |
| Bq9G9   | 3            | 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560|
| Bq10G9  | 3            | 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560| 2,560|

*B. quintana strains: Fuller, 2, URBQMLY4; 3, Oklahoma; 4, URBQPIEH2; 5, SH-perm; 6, URBQBAHH1; 7, URBQMTF17; 8, URBQMTF12; 9, URBQMLY5; 10, URBQBAHH1; 11, URBQLEIH6; 12, URBQMTF14; 13, URBQMTF15.

**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 Bq100G9.
The 16S rRNA gene sequences of Bartonella spp. have been shown to be similar but unique for each species (3, 6, 17, 21), and further research to resolve this issue is under way in our laboratory. Only a few phenotypic characteristics have been reported for Bartonella spp. The use of the MAbs would appear to be particularly useful in differentiating 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 URBQPIEH2 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.

ACKNOWLEDGMENTS

We thank Armand Tasmadjian for assistance with the experiments, P. E. Fournier for assistance with the photography, and P. Kelly for reviewing the manuscript.

REFERENCES

1. Angritt, P., S. M. Tuur, A. M. Macher, K. J. Smith, C. S. Park, F. P. Hobin, and C. Myrie-Williams. 1988. Epithelioid angiomatosis in HIV infection: neoplasm or cat-scratch disease. Lancet i:996.

2. Birles, R. I., T. G. Harrison, N. A. Saunders, and D. H. Molyneux. 1995. Proposals to unify the genera Grahamella and Bartonella, with descriptions of Bartonella taytorii comb. nov., Bartonella peromysci comb. nov., and three new species, Bartonella grahami sp. nov., Bartonella taylorii sp. nov., and Bartonella doshiiae sp. nov. Int. J. Syst. Bacteriol. 45:1–8.

3. Brenner, D. J., S. P. O’Connor, H. H. Winkler, and A. G. Steigerwalt. 1993. Proposals to unify the genera Bartonella and Rochalimaea, with descriptions of Bartonella quintana comb. nov., Bartonella vinsonii comb. nov., Bartonella henselae comb. nov., and Bartonella elizabethae comb. nov., and to remove the family Bartonellaceae from the order Rickettsiales. Int. J. Syst. Bacteriol. 43:777–786.
4. Brouqui, P., B. Lascola, V. Roux, and D. Raoult. 1999. Chronic Bartonella quintana bacteremia in homeless patients. N. Engl. J. Med. 340:184–189.

5. Cockerell, C. J., G. F. Webster, M. A. Whittow, and A. E. Friedman-Kien. 1987. Epithelioid angiomatisos: a distinct vascular disorder in patients with the acquired immunodeficiency syndrome or AIDS-related complex. Lancet ii:654–656.

6. Daly, J. S., M. G. Worthington, D. J. Brenner, C. M. Moss, D. G. Hollis, R. S. Weyant, A. G. Steigerwalt, A. M. Whitney, and D. J. Daly, J. S., M. G. Worthington, D. J. Brenner, C. M. Moss, D. G. Hollis, R. S. Cockerell, C. J., G. F. Webster, M. A. Whitlow, and A. E. Friedman-Kien. 1996. Diagnosis of 22 new cases of Bartonella endocarditis. Ann. Intern. Med. 125:646–652.

19. Philip, R. N., E. A. Casper, W. Burgdorfer, R. K. Gerlof, L. B. Hughes, and E. J. Bell. 1978. Serologic typing of Rickettsiae of the spotted fever group by microimmunofluorescence. J. Immunol. 121:1961–1968.

18. Raoult, D., P. E. Fournier, M. Drancourt, T. J. Marrie, J. Etienne, J. Cosserat, P. Cacoub, Y. Poinsignon, P. Leclercq, and A. M. Sefton. 1996. Bartonella (Rochalimaea) quintana infection in patients with chronic adenopathy, lymphoppenia, and a cat. Lancet 343:977.

20. Regnery, R. L., B. E. Anderson, J. E. C. Barridge, M. C. Rodriguez-Barradas, D. C. Jones, and J. H. Carr. 1992. Characterization of a novel Rochalimaea species, R. henselae sp. nov., isolated from blood of a febrile, human immunodeficiency virus-positive patient. J. Clin. Microbiol. 30:265–274.

22. Relman, D. A., J. S. Louiti, T. M. Smith, S. Falkow, and L. S. Tompkins. 1990. The agent of bacillary angiomatisos. An approach to the identification of uncultured pathogens. N. Engl. J. Med. 323:1573–1580.

23. Ristic, R., and J. P. Kreier. 1984. Family II. Bartonellaceae, p. 717–719. In N. R. Krieg and J. G. Holt (ed.), Bergey’s manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore, Md.

24. Roux, V., and D. Raoult. 1999. Body lice as tools for diagnosis and surveillance of reemerging diseases. J. Clin. Microbiol. 37:596–599.

25. Slater, L. N., D. W. Coody, L. K. Woolridge, and D. F. Welch. 1992. Murine antibody responses distinguish Rochalimaea henselae from Rochalimaea quintana. J. Clin. Microbiol. 30:1722–1727.

26. Spach, D. H., A. S. Kantor, M. J. Dougherty, A. M. Larson, M. B. Coley, D. J. Brenner, B. Swaminathan, G. M. Matar, D. F. Welch, R. K. Root, and W. E. Stamm. 1995. Bartonella (Rochalimaea) quintana bacteremia in inner-city patients with chronic alcoholism. N. Engl. J. Med. 332:424–428.

27. Spach, D. H., K. P. Callis, D. S. Paauw, Y. B. Houze, F. D. Schoenkecht, D. F. Welch, H. Rosen, and D. J. Brenner. 1993. Endocarditis caused by Rochalimaea quintana in a patient infected with human immunodeficiency virus. J. Clin. Microbiol. 31:692–694.

28. Vinson, J. W., and H. S. Fuller. 1961. Studies on trench fever I. Propagation of Rickettsia-like organisms from a patient’s blood. Pathol. Microbiol. 24:652–656.

29. Wong, M. T., M. J. Dolan, C. P. Lattuada, Jr., R. L. Regnery, M. L. Garcia, E. C. Mokulis, R. C. LaBarre, D. P. Ascher, J. A. Delmar, J. W. Kelly, D. R. Leigh, A. C. McRae, J. B. Reed, R. E. Smith, and G. P. Melcher. 1995. Neuroretinitis, aseptic meningitis, and lymphadenitis associated with Bartonella (Rochalimaea) henselae infection in immunocompetent patients and patients infected with human immunodeficiency virus type 1. Clin. Infect. Dis. 21:352–360.