

NOTES

An OspC-Specific Monoclonal Antibody Passively Protects Mice from Tick-Transmitted Infection by *Borrelia burgdorferi* B31

M. LAMINE MBOW,^{1*} ROBERT D. GILMORE, JR.,² AND RICHARD G. TITUS¹

Department of Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University,¹ and Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Public Health Service, U.S. Department of Health and Human Services,² Fort Collins, Colorado

Received 31 March 1999/Returned for modification 11 May 1999/Accepted 21 July 1999

A murine monoclonal antibody directed against *Borrelia burgdorferi* B31 outer surface protein C (OspC) antigen was generated by a method whereby borreliae were inoculated into the mouse via the natural transmission mode of tick feeding. Passive immunization with this antibody resulted in protection of C3H/HeJ and outbred mice from a tick-transmitted challenge infection. Immunofluorescence staining of borrelia cells indicated surface exposure of the OspC epitope reactive with the monoclonal antibody.

The outer surface protein C (OspC) of *Borrelia burgdorferi*, the causative agent of Lyme disease, elicits a protective immune response against infection in animals which have been actively immunized with the recombinant antigen (4, 9, 10). Anti-OspC activity in polyclonal serum samples from *B. burgdorferi*-infected, immune hosts has been implicated in protection (6, 7). Also, a polyclonal, monospecific anti-recombinant OspC has shown therapeutic properties when passively administered to chronically infected subjects (14). To our knowledge, however, no monoclonal antibodies (MAbs) against OspC have been documented to demonstrate protection upon passive transfer. This laboratory generated a panel of MAbs derived from a tick-borne *B. burgdorferi* antigen introduction method, rather than using an inoculum of borrelial antigens obtained from in vitro-cultivated organisms (5). One of these MAbs was directed against OspC. The present study was performed to determine if this anti-OspC MAb would passively protect experimental mice.

The generation of the MAb panel has been described elsewhere (5). Briefly, *B. burgdorferi*-infected nymphal ticks were allowed to feed on 8- to 12-week-old female BALB/cByJ mice. Infection of the mice with *B. burgdorferi* was confirmed by positive cultures derived from ear biopsy specimens (12). The mice were reinfested with *B. burgdorferi*-infected ticks 1 month later. At the end of this immunization schedule, serum samples were analyzed by enzyme-linked immunosorbent assay (ELISA) against *B. burgdorferi* whole-cell lysate, and the mouse with the highest titer was selected for hybridoma production. Three days before the cell fusion procedure, 10⁵ strain B31 low-passage-number organisms (passage 1, cultured from ticks) were injected intravenously. This boost served to enhance existing, primed B-cell polyclonal populations prior to spleen harvesting, but only those populations common to antigens expressed in both cultured and tick-transmitted *B. burgdorferi*. Spleen cells were harvested and fused with cP3x63-Ag8.653 myeloma cells by use of polyethylene glycol 1000. The

spleen cell/myeloma cell ratio was approximately 5:1. Fused cells were selected by using medium containing hypoxanthine-aminopterin-thymidine. Wells were screened by ELISA and Western blotting with low-passage-number *B. burgdorferi* B31 as the antigen. Cells from positive wells were expanded and cloned by limited dilution. The OspC specificity of one of the MAbs was determined by its immunoblot reactivity against recombinant OspC, and it was designated B5 (Fig. 1). MAb B5 was isotyped as an immunoglobulin G2a (IgG2a).

Anti-OspC IgG was purified from ascitic fluid by ammonium sulfate precipitation. Groups of test mice were injected intravenously with 100 μ l containing either 200 to 300 μ g of the anti-OspC antibody or the same amount of normal mouse IgG 1 day prior to tick infestation. Inbred mice (C3H/HeJ) and outbred mice (specific-pathogen-free mice maintained at the Division of Vector-Borne Infectious Diseases) were used in this study. One day following passive transfer of the antibody,

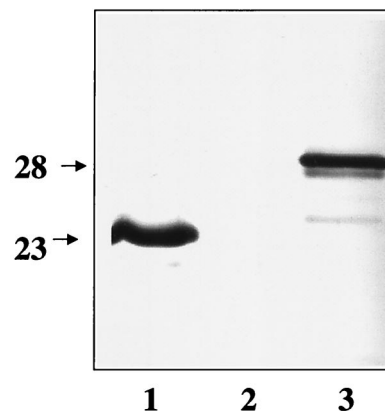


FIG. 1. Western blot demonstrating MAb B5 reactivity against OspC antigens. Lanes: 1, *B. burgdorferi* B31 lysate; 2, lysate from *Escherichia coli* harboring the plasmid expression vector pBluescript (Stratagene, La Jolla, Calif.) only; 3, *E. coli* lysate expressing OspC from pBluescript. Molecular mass markers, in kilodaltons, are indicated on the left. The recombinant OspC migrates higher than the *B. burgdorferi* OspC due to the addition of a vector-encoded fusion partner. MAb B5 was used at a dilution of 1:5,000.

* Corresponding author. Mailing address: Department of Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-1671. Phone: (970) 491-1607. Fax: (970) 491-0603. E-mail: lmbow@lamar.colostate.edu.

TABLE 1. Results of infectivity assay 4 weeks postchallenge

Antibody ^a and mouse strain	No. of mouse samples positive for infection/no. tested ^b	% Protection
Normal mouse IgG		
C3H/HeJ	4/4	0
Outbred	7/7	0
Anti-OspC MAb B5 IgG		
C3H/HeJ	0/4	100
Outbred	0/7	100

^a Antibody used for passive immunization.

^b Two assays, ear biopsy culture and Western blotting, were used, with identical results.

each mouse was infested with 10 *B. burgdorferi*-infected nymphal *Ixodes scapularis* ticks, which were allowed to feed to repletion. The B31 strain-infected tick colony has been described previously (8). To assay for infectivity, ear skin biopsy specimens were cultured 4 weeks post-tick feeding as described previously (12); also, serological bleedings were taken between 2 and 4 weeks after tick drop-off and samples were assayed by Western blotting. One positive-control mouse was passively immunized with a polyclonal anti-OspA antibody, which was known to be protective (2, 3).

All mice passively immunized with the anti-OspC MAb were protected from infection (11 of 11), whereas each mouse inoculated with control antibody was not protected (Table 1). Following feeding, replete ticks were randomly collected from the OspC-immunized inbred mice and were surface sterilized (by serial washes in 70% ethanol for 2 min, 3% hydrogen peroxide for 2 min, and sterile water for 2 min), crushed, and inoculated into Barbour-Stoenner-Kelley modified culture medium (BSK II medium). Seven of nine ticks yielded viable borreliae in culture, reflecting the 70 to 80% infection rate of the tick colony. This result ensured that the ticks placed upon the mice were indeed harboring *B. burgdorferi*. It also corroborated a result previously seen in this laboratory, that a population of borreliae survives within the tick following feeding on OspC-immunized mice (4), unlike borreliae in ticks that feed on OspA-immunized mice (3).

To assess the cell surface accessibility of the antibody, indirect immunofluorescence was performed on cultured B31 organisms as follows. Five microliters of a stationary-phase borrelial culture grown in BSK II medium was allowed to air dry on a microscope slide. The cells were heat fixed by a slight warming of the slide but were not acetone or methanol fixed. The organisms were overlaid with a 1:100 dilution of the anti-OspC B5 antibody and incubated for at least 1 h at room temperature. Following the primary incubation, the slide was

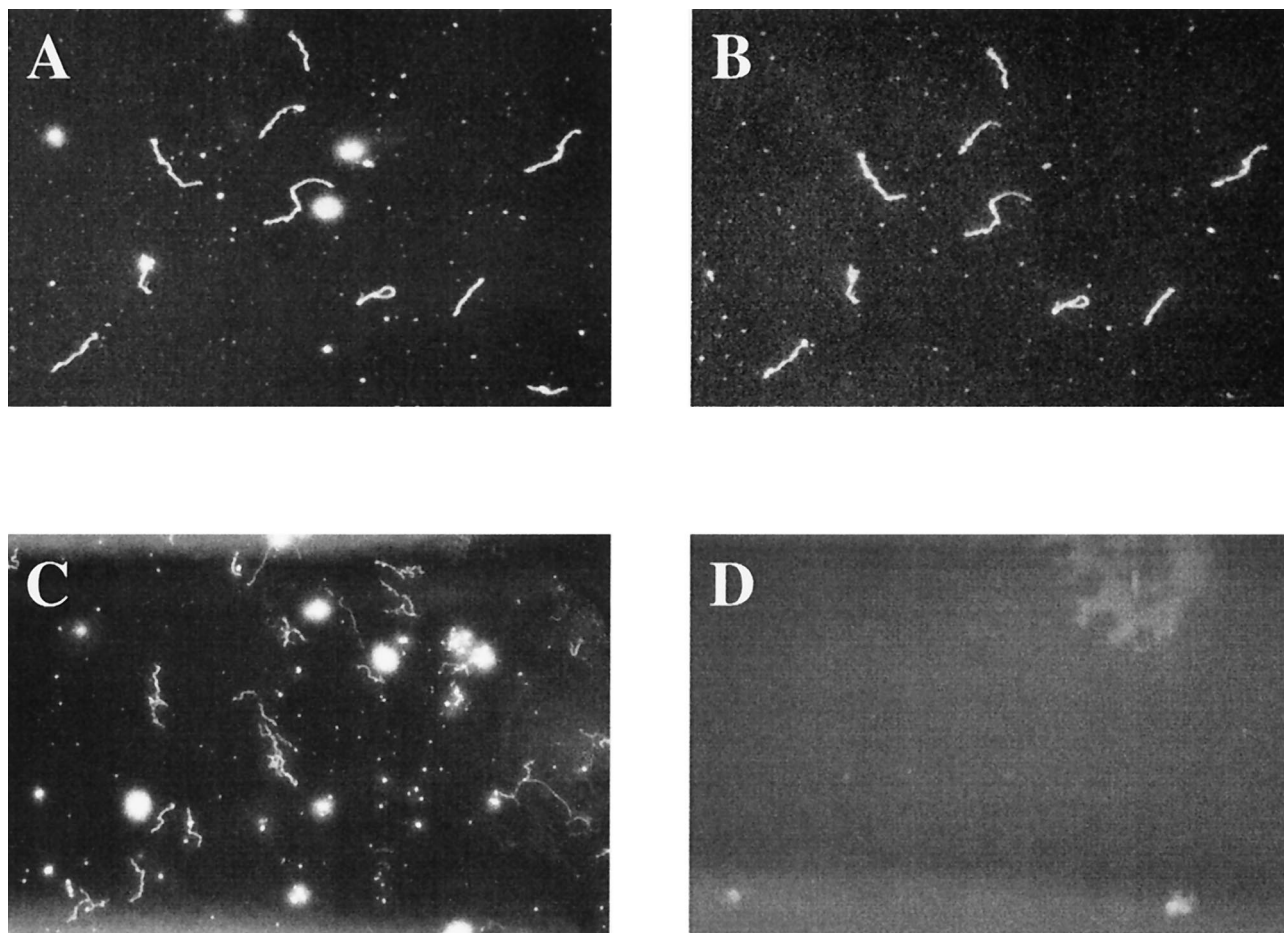


FIG. 2. Indirect immunofluorescence staining of *B. burgdorferi* B31 cultured cells labeled with anti-OspC MAb B5 or normal mouse serum. (A) Representative field under dark-field microscopy; (B) the same field as in panel A labeled with anti-OspC MAb B5; (C) a second representative field under dark-field microscopy; (D) the same field as in panel C labeled with normal mouse serum. Magnification, $\times 400$.

washed three times in phosphate-buffered saline (PBS) and allowed to air dry, followed by a secondary incubation with a 1:50 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) for 1 h at room temperature. The slide was washed three times with PBS, allowed to air dry, and overlaid with mounting medium (Kirkegaard & Perry Laboratories) and a coverslip. The finished slide was viewed under a dark-field microscope, and by epifluorescence with a fluorescein filter, at magnification of $\times 100$ and $\times 400$. The nonfixed borreliae fluoresced, indicating surface exposure of the OspC epitope reactive with this antibody (Fig. 2B). In addition, an indirect immunofluorescence assay using a MAb directed against flagellin, a non-surface-exposed antigen, gave a negative signal when nonfixed *B. burgdorferi* were used (data not shown). Normal mouse serum failed to cause the borreliae cells to fluoresce (Fig. 2D). Surface exposure of OspC epitopes in some *B. burgdorferi* strains has been speculated to correlate with protective capability (1), and the surface accessibility of the OspC epitope reactive with MAb B5 shown in Fig. 2B is consistent with that observation.

This study has demonstrated the protective efficacy of a MAb directed against the *B. burgdorferi* B31 OspC antigen. Because the protective properties of active immunization with OspC, and the fact that OspC expression is upregulated on the borrelial surface during tick feeding (11), have been well documented it is logical that a MAb generated by antigen inoculation via the natural route of tick bites would mirror the protective ability of the immunogen. Unexplained differences in the therapeutic effects of passively transferred and actively induced anti-OspC antibodies have been observed (13, 14). This MAb could be used to examine whether recognition of different epitopes is required for protection versus therapeutic clearance of infection.

We gratefully acknowledge the contributions of Rendi Murphree, Sarah Sullivan, and Steve Sviat. We express our thanks to Marc Dolan and Joe Piesman for providing ticks.

REFERENCES

1. Bockenstedt, L. K., E. Hodzic, S. Feng, K. W. Bourrel, A. de Silva, R. R. Montgomery, E. Fikrig, J. D. Radolf, and S. W. Barthold. 1997. *Borrelia burgdorferi* strain-specific Osp C-mediated immunity in mice. *Infect. Immun.* **65**:4661–4667.
2. de Silva, A. M., S. R. Telford III, L. R. Brunet, S. W. Barthold, and E. Fikrig. 1996. *Borrelia burgdorferi* OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. *J. Exp. Med.* **183**:271–275.
3. Fikrig, E., S. R. Telford III, S. W. Barthold, F. S. Kantor, A. Spielman, and R. A. Flavell. 1992. Elimination of *Borrelia burgdorferi* from vector ticks feeding on OspA-immunized mice. *Proc. Natl. Acad. Sci. USA* **89**:5418–5421.
4. Gilmore, R. D., Jr., K. J. Kappel, M. C. Dolan, T. R. Burkot, and B. J. Johnson. 1996. Outer surface protein C (OspC), but not P39, is a protective immunogen against a tick-transmitted *Borrelia burgdorferi* challenge: evidence for a conformational protective epitope in OspC. *Infect. Immun.* **64**:2234–2239.
5. Gilmore, R. D., Jr., and M. L. Mbow. 1998. A monoclonal antibody generated by antigen inoculation via tick bite is reactive to the *Borrelia burgdorferi* Rev protein, a member of the 2.9 gene family locus. *Infect. Immun.* **66**:980–986.
6. Hughes, C. A., S. M. Engstrom, L. A. Coleman, C. B. Kodner, and R. C. Johnson. 1993. Protective immunity is induced by a *Borrelia burgdorferi* mutant that lacks OspA and OspB. *Infect. Immun.* **61**:5115–5122.
7. Kurtji, T. J., U. G. Munderloh, C. A. Hughes, S. M. Engstrom, and R. C. Johnson. 1996. Resistance to tick-borne spirochete challenge induced by *Borrelia burgdorferi* strains that differ in expression of outer surface proteins. *Infect. Immun.* **64**:4148–4153.
8. Piesman, J. 1993. Standard system for infecting ticks (Acari: Ixodidae) with the Lyme disease spirochete, *Borrelia burgdorferi*. *J. Med. Entomol.* **30**:199–203.
9. Preac-Mursic, V., B. Wilske, E. Patouris, S. Jauris, G. Will, E. Soutschek, S. Rainhardt, G. Lehnert, U. Klockmann, and P. Mehraein. 1992. Active immunization with pC protein of *Borrelia burgdorferi* protects gerbils against *B. burgdorferi* infection. *Infection* **20**:342–349.
10. Probert, W. S., and R. B. LeFebvre. 1994. Protection of C3H/HeN mice from challenge with *Borrelia burgdorferi* through active immunization with OspA, OspB, or OspC, but not with OspD or the 83-kilodalton antigen. *Infect. Immun.* **62**:1920–1926.
11. Schwan, T. G., J. Piesman, W. T. Golde, M. C. Dolan, and P. A. Rosa. 1995. Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. *Proc. Natl. Acad. Sci. USA* **92**:2909–2913.
12. Sinsky, R. J., and J. Piesman. 1989. Ear punch biopsy method for detection and isolation of *Borrelia burgdorferi* from rodents. *J. Clin. Microbiol.* **27**:1723–1727.
13. Zhong, W., L. Gern, T. Stehle, C. Museteanu, M. Kramer, R. Wallich, and M. M. Simon. 1999. Resolution of experimental and tick-borne *Borrelia burgdorferi* infection in mice by passive, but not active immunization using recombinant OspC. *Eur. J. Immunol.* **29**:946–957.
14. Zhong, W., T. Stehle, C. Museteanu, A. Siebers, L. Gern, M. Kramer, R. Wallich, and M. M. Simon. 1997. Therapeutic passive vaccination against chronic Lyme disease in mice. *Proc. Natl. Acad. Sci. USA* **94**:12533–12538.

Editor: R. N. Moore