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Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding

(*Ixodes scapularis*/Lyme disease/blood meal/outer surface protein C/temperature)

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ABSTRACT **Lyme disease spirochetes, *Borrelia burgdorferi sensu lato*, are maintained in zoonotic cycles involving ticks and small mammals. In unfed ticks, the spirochetes produce one outer surface protein, OspA, but not OspC. During infection in mammals, immunological data suggest that the spirochetes have changed their surface, now expressing OspC but little or no OspA. We find by *in vitro* growth experiments that this change is regulated in part by temperature; OspC is produced by spirochetes at 32–37°C but not at 24°C. Furthermore, spirochetes in the midgut of ticks that have fully engorged on mice now have OspC on their surface. Thus two environmental cues, an increase in temperature and tick feeding, trigger a major alteration of the spirochetal outer membrane. This rapid synthesis of OspC by spirochetes during tick feeding may play an essential role in the capacity of these bacteria to successfully infect mammalian hosts, including humans, when transmitted by ticks.**

Many infectious agents pathogenic in humans are maintained in natural zoonotic cycles involving wild vertebrates and obligate blood-feeding arthropods (1, 2). Although much is known about the clinical description and diagnosis of these human diseases, the physiological and morphological adaptations of these agents, especially bacteria, while in their arthropod vectors are not well understood. Several non-vector-borne bacterial pathogens, including species of *Salmonella, Shigella*, and *Bordetella*, display an impressive repertoire of adaptive molecular responses to environmental signals on entry into mammalian hosts (3–5). Specific changes by bacterial pathogens in arthropod vectors during feeding on blood have not been described. Identifying such events would broaden our knowledge of how these agents are perpetuated and transmitted in nature and assist in the development of effective vaccines and diagnostic tests.

Borrelia burgdorferi is one of at least three closely related species of spirochetes that cause a spectrum of clinical syndromes in humans, collectively called Lyme disease or Lyme borreliosis (6–8). These spirochetes are maintained in zoonotic cycles involving a diversity of wild mammals and ticks primarily in the genus *Ixodes* (9). These microbes' adaptation to tick and mammalian environments likely involves very different surface components so as to ensure their transmission and survival in two very different hosts. Several lipoproteins have been described on the surface of *B. burgdorferi* (10–16), some of which are variably expressed during serial passage in culture (17–19). The apparent flexibility in the spirochete's synthesis of some outer surface proteins (Osps) *in vitro* may have relevance to its alternation of hosts. Previous studies demonstrate that *B. burgdorferi* produces one surface protein, OspA, and likely OspB, in the midgut of *Ixodes* ticks that have not yet engorged on blood (10). However, it is unclear how long spirochetes continue to produce this protein after entering mammalian hosts because few animals, including humans, make antibodies to this protein during infection (20–22). Yet, another surface protein, OspC, stimulates an early antibody response in humans (23, 24), although its expression in ticks is unknown. In this report we demonstrate that the Lyme disease spirochete alters its outer surface during tick feeding and that this switch is also controlled, in part, by an increase in temperature.

MATERIALS AND METHODS

Bacterial Isolates and Tick Infections. *B. burgdorferi* B31 (ATCC 35210), the prototype strain, originated from a female *Ixodes scapularis* collected from Shelter Island, New York (6); *B. burgdorferi* JD-1 originated from a pool of nymphal *I. scapularis* collected at Cranes' Beach, Ipswich, Massachusetts (25); *B. burgdorferi* CA-28-91 originated from a pool of 10 *Ixodes pacificus* collected in Kern County, California (26). *I. scapularis* nymphs from a clean laboratory colony were infected with either the JD-1 or B31 strain of *B. burgdorferi* by allowing them to feed as uninfected larvae on mice infected with one of these two strains. These ticks were held at 21–22°C and 95% relative humidity and allowed to molt to nymphs. Unfed nymphs were then examined for spirochetal infection of the midgut.

Indirect Immunofluorescence Microscopy. Tick midguts were smeared onto a glass slide, dried gently by flame, and fixed for 10 min with acetone. Cultured spirochetes were removed from the BSK-II medium (27) by centrifugation, rinsed twice in PBS, and mixed with washed sheep red blood cells. Thin smears of these suspensions were made on glass slides, air dried, and fixed with methanol. Spirochetes were stained for OspA using monoclonal antibody H5332 (10) followed by goat anti-mouse fluorescein isothiocyanate and for OspC using a rabbit polyclonal antiserum specific for this protein (26) followed by goat anti-rabbit fluorescein isothiocyanate. These preparations were examined by epifluorescence microscopy.

For double staining of cultured spirochetes, we used the B31 strain of *B. burgdorferi* derived from an infected tick (see below) following growth at either 24°C or 37°C. Spirochetes fixed on a glass slide were first incubated with a mixture of monoclonal antibody H5332 (anti-OspA) and the rabbit anti-OspC antiserum. The slides were washed with PBS and then incubated with a mixture of goat anti-mouse fluorescein isothiocyanate and goat anti-rabbit rhodamine isothiocyanate. The same fields of view were examined by epifluorescence microscopy using filters specific for fluorescein or rhodamine to compare staining of individual spirochetes.

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Abbreviation: Osp, outer surface protein.

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Isolation of Spirochetes from Ticks. The midgut from one unfed *I. scapularis* nymph infected with JD-1 and one midgut from a tick infected with B31 were dissected out, and each was placed in a culture tube containing 9 ml of BSK-II medium (27) with rifampin (50 μg/ml), phosphomycin (100 μg/ml), and amphotericin B (10 μg/ml). After 5 days of incubation at 33°C, the medium with spirochetes and contaminating bacteria were filtered through a 0.22-μm Millex-GS disposable filter (Millipore) and passaged into new medium. These cultures provided pure isolates of both strains for subsequent analysis.

Mouse Infections. All mice were adult ICR outbred. Six mice were infected by tick bite with *B. burgdorferi* B31; serum samples from these mice were collected 17 days after the ticks had completed feeding. Nine mice were infected with *B. burgdorferi* JD-1: seven mice by intraperitoneal inoculation of infected midguts from partially engorged ticks and two mice by tick bite. Serum samples from the injected mice were collected 28 days later. Serum samples from the two mice infected by tick bite were collected 28 and 34 days after ticks had finished feeding. All animal care was in accordance with National Institutes of Health institutional guidelines.

SDS/PAGE and Immunoblotting. Whole-cell lysates of spirochetes were electrophoresed in an SDS/12.5% PAGE gel using the Laemmli buffer (28) and a vertical gel electrophoresis system (Bethesda Research Laboratories/GIBCO). Proteins were visualized by staining with Coomassie brilliant blue.

Serum samples from mice were tested by immunoblot with lysates of *B. burgdorferi* JD-1 and B31 isolated from the single ticks and *Escherichia coli* DH5-α transformed with recombinant plasmids containing either the *ospC* or *ospA* gene of *B. burgdorferi*. The *ospC* gene of *B. burgdorferi* 297 was expressed as a fusion protein with the maltose binding protein using the pMAL expression vector, which was kindly provided by William Probert and Rance LeFebvre (University of California, Davis). The *ospA* gene from *B. burgdorferi* Sh-2-82 was expressed from its own promoter in pUC19. After electrophoresis, whole-cell lysates were blotted onto nitrocellulose membranes using the Towbin buffer system (29) and a Trans-Blot cell (Bio-Rad) following the instructions of the manufacturer. The membranes were blocked overnight at room temperature with TSE/Tween (50 mM Tris, pH 7.4/150 mM NaCl/5 mM EDTA/0.05% Tween 20) and subsequently incubated with either the serum samples from the 15 mice infected with the JD-1 or B31 strains of *B. burgdorferi*, rabbit anti-OspC antiserum, or anti-flagellin monoclonal antibody H9724 (30). Bound antibodies were detected directly with ^{125}I-labeled protein A autoradiography as described (20).

RESULTS AND DISCUSSION

We examined the differential expression of Osps by spirochetes in ticks by first infecting larval *I. scapularis* with *B. burgdorferi*. After allowing the ticks to molt to nymphs, spirochetes in the ticks' midgut were examined by indirect immunofluorescence for the presence of OspA or OspC (Fig. 1). Spirochetes positive for OspA were abundant in 100% of 22 unfed ticks (14 infected with JD-1 and 8 infected with B31) (Fig. 1*A*). However, no spirochetes from 25 additional ticks were positive for OspC (15 infected with JD-1 and 10 infected with B31) (Fig. 1*B*), although this protein was readily detectable in a cultured strain using the same antiserum (Fig. 1*C*). When the OspC-negative smears were stained again for OspA, all 25 were heavily infected with spirochetes (Fig. 1*D*). These results demonstrated that *B. burgdorferi* produced OspA but not OspC in the midgut of ticks that had not yet fed on blood.

One explanation for our inability to detect OspC in the ticks was that these spirochetes were constitutively unable to produce this protein. To examine that possibility, spirochetes were isolated from single nymphal *I. scapularis* from the same cohorts of ticks, passaged *in vitro*, and examined by SDS/PAGE and immunoblotting. This demonstrated that spirochetes of both strains expressed OspC after only two passages at 33°C (Figs. 2 and 3*C*).

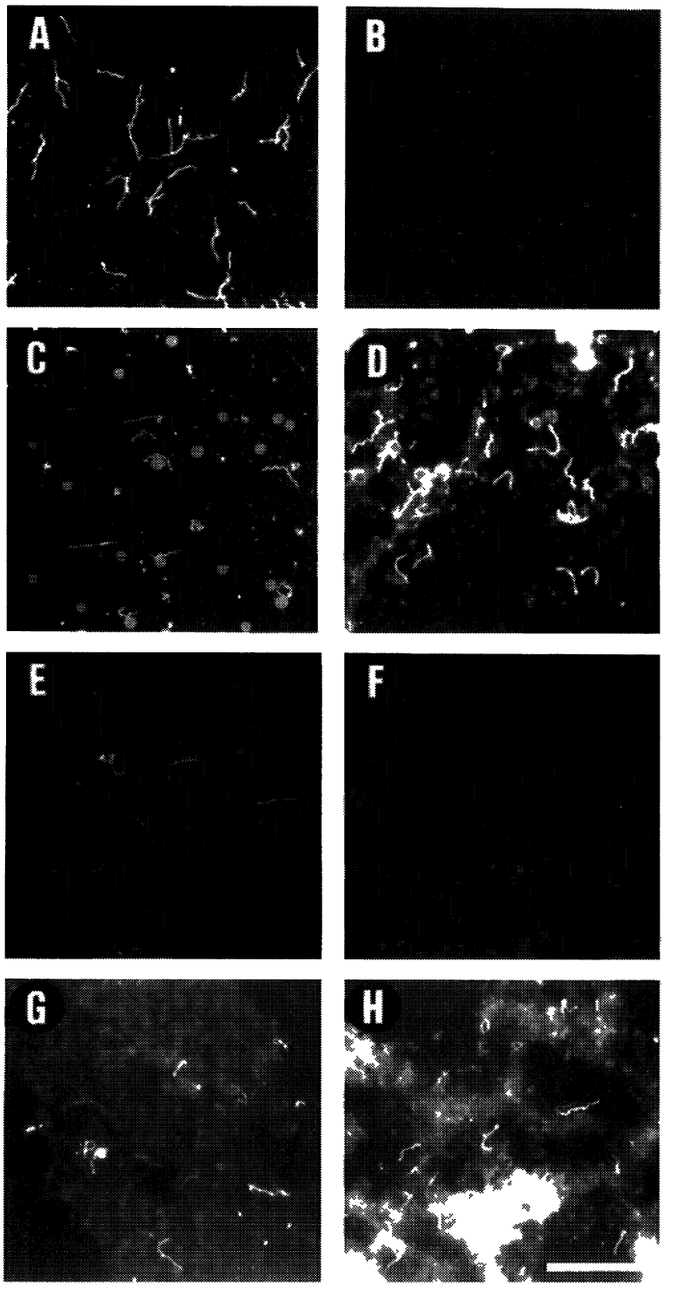

FIG. 1. Indirect immunofluorescence staining of *B. burgdorferi* in ticks or culture for the presence of OspA or OspC. (*A*) Midgut of an unfed tick infected with strain JD-1 stained for OspA. (*B*) Midgut of an unfed tick infected with strain JD-1 stained for OspC. (*C*) Cultured spirochetes of strain CA-28-91 stained for OspC. This is a high-OspC-producing strain, five passages after isolation from *I. pacificus* ticks collected in California and is included here and in Figs. 2 and 3 as a positive control. (*D*) Midgut of an unfed tick infected with strain JD-1, which was negative when first stained for OspC but positive when restained for OspA. (*E*) Cultured spirochetes of strain B31 derived from our infected ticks, grown at 37°C, and stained for OspC. (*F*) Cultured spirochetes of strain B31 derived from our infected ticks, grown at 24°C, and stained for OspC. (*G*) Midgut of a fully engorged tick infected with strain JD-1 stained for OspC. (*H*) Midgut of a fully engorged tick infected with strain JD-1 stained for OspA. (Bar = 40 μm.)

Next we sought evidence that OspC and OspA were produced by these spirochetes during infection in mammals. Because Lyme disease spirochetes are rarely detectable in

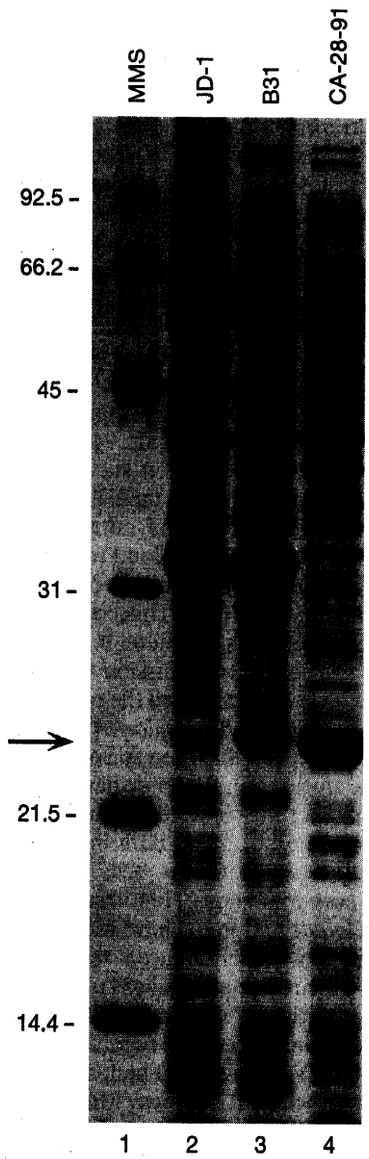

FIG. 2. Presence of OspC (arrow) in strains JD-1 (lane 2) and B31 (lane 3) of *B. burgdorferi* detected after only two passages subsequent to their isolation from single ticks and in strain CA-28-91, passage 5 (lane 4). Proteins were stained with Coomassie brilliant blue, and molecular mass standards (lane 1) are shown in kilodaltons.

mammals by direct staining, we examined antisera from 15 ICR outbred mice infected with spirochetes by tick bite or by inoculation of midguts from partially engorged, infected ticks. Antisera from all mice had strong antibody responses to OspC in immunoblots using spirochetal lysates (Fig. 3), as well as to recombinant OspC produced in *E. coli* (data not shown). Immunoblots of spirochetal lysates also demonstrated more antibody bound to OspC of the homologous versus heterologous strains. This immunological specificity for the OspC of spirochetes with which the mice were infected (Fig. 3) is explained, in part, by significant levels of sequence variation in the *ospC* gene from North American strains of *B. burgdorferi* (31). None of the sera had antibodies to OspA detectable by immunoblots using the spirochetal lysates or recombinant OspA produced in *E. coli*. These data indicated that *B. burgdorferi* produced OspC but little or no OspA during early infection in mammals.

B. burgdorferi synthesized OspC in culture at 33°C and in mice but not in ticks that had not yet fed on blood. Would a change in temperature, as happens when spirochetes transfer between tick and mammal hosts, influence the synthesis of OspC *in vitro*? Spirochetes were passaged into new culture medium, grown at 24°C or 37°C, and then examined by

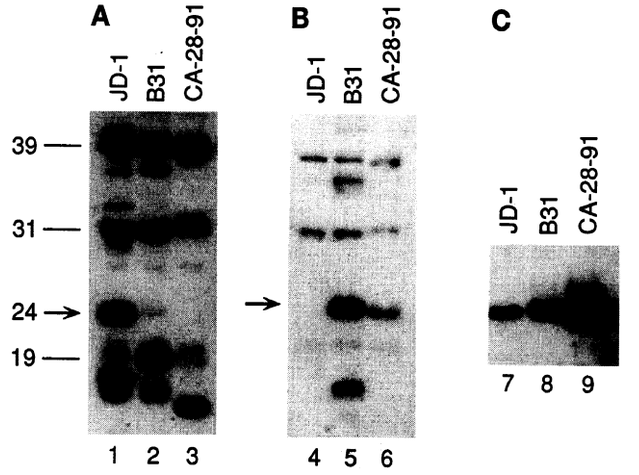

FIG. 3. Presence of anti-OspC antibodies (arrows) in mice infected with the JD-1 or B31 strain of *B. burgdorferi*. Bound antibodies were detected directly with ^{125}I-labeled protein A. Estimates of molecular mass are shown on the left in kilodaltons. (*A*) Antiserum from mouse M1018 infected 28 days previously with strain JD-1 by intraperitoneal inoculation of a midgut from a partially engorged infected tick. (*B*) Antiserum from mouse M3444 infected 17 days previously with strain B31 by tick bite. (*C*) Control antiserum from a rabbit hyperimmunized with gel-purified OspC. The antiserum tested from the other mice infected with JD-1 or B31 showed nearly identical patterns of antibody binding as shown by the two examples presented for each group in *A* and *B*. Note the much stronger binding of antibodies to OspC in the homologous strains (lanes 1 and 5) compared to the much weaker affinity of anti-OspC antibodies to this protein in the heterologous strains (lanes 2, 3, 4, and 6). The top bands in lanes 1–6 represent antibodies binding to the p39 antigen.

SDS/PAGE and immunoblotting. Both strains continued to produce OspC when grown at 37°C but very little at 24°C (data not shown). When spirochetes were passaged again into new culture medium and grown at the alternate temperature, the amount of OspC detected in the lysates changed (Fig. 4): spirochetes grown first at 24°C and then at 37°C greatly increased their OspC while spirochetes grown first at 37°C and then at 24°C now had no detectable OspC (Fig. 1 *E* and *F*; Fig. 4). The double staining of spirochetes by indirect immunofluorescence with both anti-OspA and anti-OspC antibodies revealed that the population of spirochetes was a phenotypic mixture after temperature elevation to 37°C: some spirochetes had only OspA, some had only OspC, and some had both proteins. The amount of another protein, flagellin, detected in the same lysates did not vary with temperature (Fig. 4). These results corroborated our observations *in vivo* and demonstrated that the synthesis of OspC was regulated, at least in part, by temperature.

Ticks attached and feeding would experience both an increase in temperature and the influx of fresh blood into the midgut where spirochetes reside. Midguts from 10 *I. scapularis* nymphs infected with the JD-1 strain were examined immediately after the ticks had engorged in 3 days on normal mice, to determine if this influenced the production of OspC by the spirochetes. Five smears were stained for OspC and five smears were stained for OspA. In striking contrast to the spirochetes in unfed ticks, spirochetes in all of the tick smears stained for OspC were positive (Fig. 1*G*), as were all of the smears stained for OspA (Fig. 1*H*). However, the incubation of 23 unfed, infected *I. scapularis* nymphs at 37°C for either 24 hr, 72 hr, or 144 hr (6 days) did not stimulate the spirochetes to produce OspC detectable by indirect immunofluorescence. These observations demonstrated that during tick feeding, but not after an increase in temperature of the ticks alone, spirochetes in the midgut synthesized OspC.

Many species of pathogenic bacteria possess proteins, some identified as virulence factors, that are synthesized at different

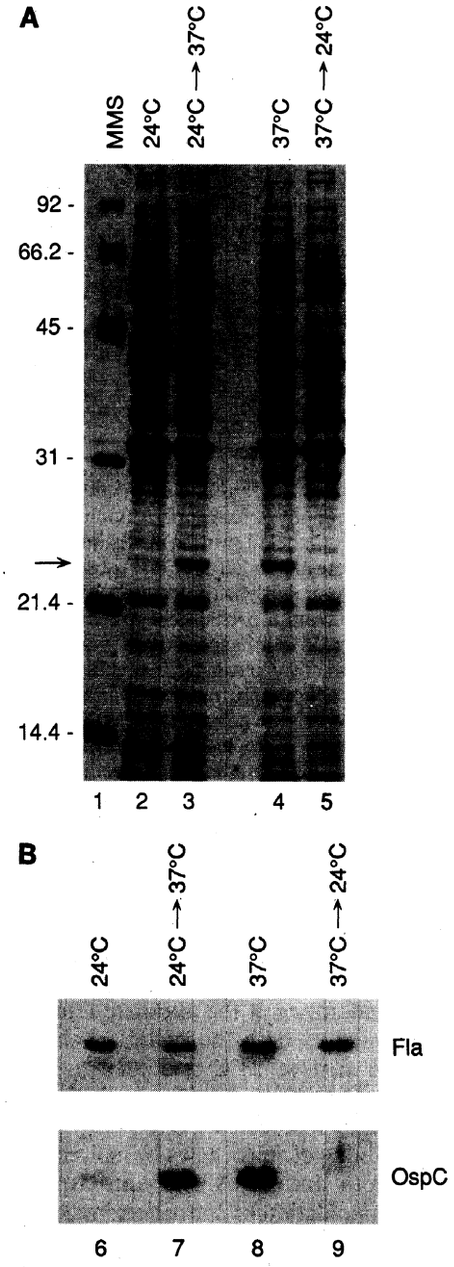

FIG. 4. Regulation in the synthesis of OspC by strain B31 of *B. burgdorferi* with switches in temperature. (*A*) Whole-cell lysates of spirochetes were examined by SDS/PAGE prior to and after temperature switches during *in vitro* growth. OspC (arrow) was turned on when spirochetes were transferred from 24°C to 37°C (lanes 2 and 3) and turned off when transferred from 37°C to 24°C (lanes 4 and 5). Molecular mass standards (lane 1) are shown in kilodaltons. (*B*) Immunoblot analysis with specific antiserum (OspC) confirmed that the temperature-regulated protein in the stained gel was OspC (lanes 6–9). The amount of flagellin (Fla) did not appear to vary at the different temperatures after blotting with the anti-flagellin monoclonal antibody H9724.

rates depending on the temperature and other environmental changes (3–5, 32, 33). For the Lyme disease spirochete, what is the biological significance for the rapid change on this bacterium's surface during tick feeding? In unfed *I. scapularis*, spirochetes with OspA, but not OspC, on their surface are primarily restricted to the tick's midgut (34). Although spirochetes may be very abundant in midguts of unfed ticks, they fail to cause infections when experimentally inoculated in rodents (35). *I. scapularis* feed for 3–11 days depending on the stage of the tick (36), although most nymphs become replete in 3–6 days. Also, *B. burgdorferi* is rarely if ever transmitted by ticks to mammals during the first 2 days of attachment and feeding (25). From 2 days onwards, however, the frequency of transmission of spirochetes by ticks increases dramatically. Interestingly, the direct inoculation of infected midguts from ticks having fed for 3 days or more causes infections in mammals; these spirochetes now have OspC on their surface.

The delay in transmission of *B. burgdorferi* during early tick feeding has been explained by the location of spirochetes restricted primarily to the midgut of unfed ticks. During tick feeding the spirochetes multiply, disseminate through the midgut wall to the hemolymph, invade the salivary glands, and are transmitted to mammals via tick saliva (25, 37–42). This entire phenomenon takes time, during which both ticks and spirochetes first warm to ≈37°C when ticks attach to the host's skin (T.G.S., unpublished data). The *ospC* gene, unlike genes encoding other surface proteins of this spirochete, is located on a supercoiled plasmid (43, 44). Temperature can influence the topology of superhelical plasmids, which in turn can affect the expression of genes located on such molecules (45, 46). Also, during feeding, blood enters the ticks's midgut, providing a milieu of undefined signals and nutrients to the resident spirochetes. Erythrocytes in the blood meal begin to be lysed after 2 days of feeding by a hemolysin only now produced in the midgut of *I. scapularis* (47). This corresponds temporally with the synthesis of OspC, spirochetes in the midgut becoming infectious, and spirochete transmission via saliva.

Other studies have demonstrated the presence of heat shock and heat stress proteins in *B. burgdorferi* (48, 49) and variable immunoreactivity of proteins when spirochetes were grown at different temperatures (50). While we have demonstrated here that the expression of the *ospC* gene is influenced by temperature, the upregulation of OspC both in the feeding tick and in BSK-II medium incubated at 37°C (also at 32°C–35°C) suggests that some component of blood is also essential. Purine synthesis genes (*guaA* and *guaB*) have recently been identified in *B. burgdorferi*, immediately adjacent to the *ospC* gene on the spirochete's 26-kb circular plasmid (51). Whether an increase in purine concentration in mammalian blood as it is digested in the tick's midgut influences the regulation of OspC remains to be studied. Many years ago the phenomenon of reactivation was described for the agent of Rocky Mountain spotted fever, *Rickettsia rickettsii*, in its tick vector, *Dermacentor andersoni* (52, 53), in which the virulence of this bacterium in unfed ticks was enhanced in guinea pigs by either first warming the infected ticks or allowing the ticks to begin feeding prior to experimental inoculation. To date, however, neither the genetic basis of this reactivation nor any molecular determinants associated with it have been identified. The synthesis of OspC may be essential for infectivity of *B. burgdorferi* in mammals following transmission by ticks. This conversion on the spirochete's surface actually begins while the bacterium is still in the tick's midgut. The fact that spirochete-infected midguts from partially engorged ticks are infectious to mammals, whereas infected midguts from unfed ticks are not (35), also suggests that OspC is a critical factor for spirochete infectivity. Some cultured isolates that produce less OspC appear to be less infectious in mice or rabbits compared to other isolates that produce more of this protein when these bacteria were experimentally injected into these animals (54, 59). This surface protein may also be required for spirochete dissemination through the wall of the tick's midgut, a prerequisite for transmission via tick saliva. Additionally, homologs to the *ospC* gene or proteins that bind anti-OspC antiserum have been identified recently in other species of *Borrelia* that are transmitted by soft ticks in the genera *Ornithodoros* and *Argas* (55–58). This implies that these related proteins may have an important function broadly conserved within the genus *Borrelia*. When a genetic exchange system for these spirochetes becomes available and specific genes can be inactivated, our hypothesis concerning the role of OspC for this bacterium's infectiousness can be tested.

CONCLUSION

In this report we demonstrate that Lyme disease spirochetes change their cell surface during tick feeding by producing OspC. To our knowledge, this is the first time that a tick-borne bacterial pathogen has been shown to alter its expression of a specific surface component during tick feeding. Further investigations of the adaptive responses that spirochetes and other arthropod-borne bacteria have evolved to allow for their alternate infections in vector and vertebrate hosts should be rewarding.

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1. James, M. T. & Harwood, R. F. (1969) *Herms's Medical Entomology* (Macmillan, Toronto), 6th Ed.
2. Theiler, M. & Downs, W. G. (1973) *The Arthropod-Borne Viruses of Vertebrates* (Yale Univ. Press, New Haven, CT).
3. Finlay, B. R. & Falkow, S. (1989) *Microbiol. Rev.* **53,** 210–230.
4. Miller, J. F., Mekalanos, J. J. & Falkow, S. (1989) *Science* **243,** 916–922.
5. Mekalanos, J. J. (1992) *J. Bacteriol.* **174,** 1–7.
6. Burgdorfer, W., Barbour, A. G., Hayes, S. F., Benach, J. L., Grunwaldt, E. & Davis, J. P. (1982) *Science* **216,** 1317–1319.
7. Steere, A. C., Grodzicki, R. L., Kornblatt, A. N., Craft, J. E., Barbour, A. G., Burgdorfer, W., Schmid, G. P., Johnson, E. & Malawista, S. E. (1983) *N. Engl. J. Med.* **308,** 733–740.
8. Johnson, R. C., Schmid, G. P., Hyde, F. W., Steigerwalt, A. G. & Brenner, D. J. (1984) *Int. J. Syst. Bacteriol.* **34,** 496–497.
9. Lane, R. S., Piesman, J. & Burgdorfer, W. (1991) *Annu. Rev. Entomol.* **36,** 587–609.
10. Barbour, A. G., Tessier, S. L. & Todd, W. J. (1983) *Infect. Immun.* **41,** 795–804.
11. Barbour, A. G., Tessier, S. L. & Hayes, S. F. (1984) *Infect. Immun.* **45,** 94–100.
12. Wilske, B., Preac-Mursic, V., Schierz, G. & Busch, K. V. (1986) *Zentralbl. Bakteriol. Mikrobiol. Hyg. A* **263,** 92–102.
13. Norris, S. J., Carter, C. J., Howell, J. K. & Barbour, A. G. (1992) *Infect. Immun.* **60,** 4662–4672.
14. Wallich, R., Simon, M. M., Hofmann, H., Moter, S. E., Schaible, U. E. & Kramer, M. D. (1993) *Infect. Immun.* **61,** 4158–4166.
15. Brandt, M. E., Riley, B. S., Radolf, J. D. & Norgard, M. V. (1990) *Infect. Immun.* **58,** 983–991.
16. Lam, T. T., Nguyen, T.-P. K., Montgomery, R. R., Kantor, F. S., Fikrig, E. & Flavell, R. A. (1994) *Infect. Immun.* **62,** 290–298.
17. Bundoc, V. G. & Barbour, A. G. (1989) *Infect. Immun.* **57,** 2733–2741.
18. Margolis, N. & Rosa, P. A. (1993) *Infect. Immun.* **61,** 2207–2210.
19. Schwan, T. G. & Burgdorfer, W. (1987) *J. Infect. Dis.* **156,** 852–853.
20. Schwan, T. G., Kime, K. K., Schrumpf, M. E., Coe, J. E. & Simpson, W. J. (1989) *Infect. Immun.* **57,** 3445–3451.
21. Golde, W. T., Kappel, K. J., Dequesne, G., Feron, C., Plainchamp, D., Capiau, C. & Lobet, Y. (1994) *Infect. Immun.* **62,** 2625–2627.
22. Kalish, R. A., Leong, J. M. & Steere, A. C. (1993) *Infect. Immun.* **61,** 2774–2779.
23. Padula, S. J., Sampieri, A., Dias, F., Szczepanski, A. & Ryan, R. W. (1993) *Infect. Immun.* **61,** 5097–5105.
24. Fung, B. P., McHugh, G. L., Leong, J. M. & Steere, A. C. (1994) *Infect. Immun.* **62,** 3213–3221.
25. Piesman, J., Mather, T. N., Sinsky, R. J. & Spielman, A. (1987) *J. Clin. Microbiol.* **25,** 557–558.
26. Schwan, T. G., Schrumpf, M. E., Karstens, R. H., Clover, J. R., Wong, J., Daugherty, M., Struthers, M. & Rosa, P. A. (1993) *J. Clin. Microbiol.* **31,** 3096–3108.
27. Barbour, A. G. (1984) *Yale J. Biol. Med.* **57,** 71–75.
28. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
29. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
30. Barbour, A. G., Hayes, S. F., Heiland, R. A., Schrumpf, M. E. & Tessier, S. L. (1986) *Infect. Immun.* **52,** 549–554.
31. Margolis, N., Hogan, D., Cieplak, W., Jr., Schwan, T. G. & Rosa, P. A. (1994) *Gene* **143,** 105–110.
32. Hoe, N. P., Minion, F. C. & Goguen, J. D. (1992) *J. Bacteriol.* **174,** 4275–4286.
33. Hromockyj, A. E., Tucker, S. C. & Maurelli, A. T. (1992) *Mol. Microbiol.* **6,** 2113–2124.
34. Burgdorfer, W., Hayes, S. F. & Benach, J. L. (1988) *Ann. N.Y. Acad. Sci.* **539,** 172–179.
35. Piesman, J. (1993) *J. Infect. Dis.* **167,** 1082–1085.
36. Krinsky, W. L. (1979) *J. Med. Entomol.* **16,** 354–355.
37. Piesman, J., Maupin, G. O., Campos, E. G. & Happ, C. M. (1991) *J. Infect. Dis.* **163,** 895–897.
38. Ribeiro, J. M. C., Mather, T. N., Piesman, J. & Spielman, A. (1987) *J. Med. Entomol.* **24,** 201–205.
39. Benach, J. L., Coleman, J. L., Skinner, R. A. & Bosler, E. M. (1987) *J. Infect. Dis.* **155,** 1300–1306.
40. Piesman, J., Oliver, J. R. & Sinsky, R. J. (1990) *Am. J. Trop. Med. Hyg.* **42,** 352–357.
41. Zung, J. L., Lewengrub, S., Rudzinska, M. A., Spielman, A., Telford, S. R. & Piesman, J. (1989) *Can. J. Zool.* **67,** 1737–1748.
42. Gern, L., Zhu, Z. & Aeschlimann, A. (1990) *Ann. Parasitol. Hum. Comp.* **65,** 89–93.
43. Marconi, R. T., Samuels, D. S. & Garon, C. F. (1993) *J. Bacteriol.* **175,** 926–932.
44. Sadziene, A., Wilske, B., Ferdows, M. S. & Barbour, A. G. (1993) *Infect. Immun.* **61,** 2192–2195.
45. Dorman, C. J. (1991) *Infect. Immun.* **59,** 745–749.
46. Rohde, J. R., Fox, J. M. & Minnich, S. A. (1994) *Mol. Microbiol.* **12,** 187–199.
47. Ribeiro, J. M. C. (1988) *J. Parasitol.* **74,** 532–537.
48. Cluss, R. G. & Boothby, J. T. (1990) *Infect. Immun.* **58,** 1038–1042.
49. Carreiro, M. M., Laux, D. C. & Nelson, D. R. (1990) *Infect. Immun.* **58,** 2186–2191.
50. Schwan, T. G. & Simpson, W. J. (1991) *Scand. J. Infect. Dis. Suppl.* **77,** 94–101.
51. Margolis, N., Hogan, D., Tilly, K. & Rosa, P. A. (1994) *J. Bacteriol.* **176,** 6427–6432.
52. Spencer, R. R. & Parker, R. R. (1923) *Public Health Rep.* **38,** 333–339.
53. Spencer, R. R. & Parker, R. R. (1924) *Public Health Rep.* **39,** 3027–3040.
54. Lane, R. S., Brown, R. N., Piesman, J. & Peavey, C. A. (1994) *J. Med. Entomol.* **31,** 417–424.
55. Marconi, R. T., Samuels, D. S., Schwan, T. G. & Garon, C. F. (1993) *J. Clin. Microbiol.* **31,** 2577–2583.
56. Wilske, B., Preac-Mursic, V., Jauris, S., Hofmann, A., Pradel, I., Soutschek, E., Schwab, E., Will, G. & Wanner, G. (1993) *Infect. Immun.* **61,** 2182–2191.
57. Margolis, N., Hogan, D., Cieplak, W., Jr., Schwan, T. G. & Rosa, P. A. (1994) *Gene* **143,** 105–110.
58. Carter, C. J., Bergström, S., Norris, S. J. & Barbour, A. G. (1994) *Infect. Immun.* **62,** 2792–2799.
59. Masuzawa, T., Kurita, T., Kawabata, H. & Yanagihara, Y. (1994) *FEMS Microbiol. Lett.* **123,** 319–324.