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Delineating the Requirement for the *Borrelia burgdorferi* Virulence Factor OspC in the Mammalian Host

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We previously demonstrated that outer surface protein C (OspC) of *Borrelia burgdorferi* is essential for establishing mammalian infection. However, the role of OspC in mammalian infection is unknown. Here, we report experiments designed to distinguish between two models of OspC function in the mammalian host: (i) OspC fulfills an essential physiological role for growth and host adaptation or (ii) OspC provides a protective role for evasion of components of the innate immune response. We found that a *B. burgdorferi ospC* mutant, previously demonstrated to be noninfectious in both immunocompetent and SCID mice, could survive in the relatively immune-privileged environment of dialysis membrane chambers implanted within the peritoneum of a rat. The *ospC* mutant also adapts to the mammalian environment, as determined by the protein profiles of the chamber-cultivated spirochetes. Therefore, OspC does not appear to provide a physiological function for the survival of *B. burgdorferi* within the mammalian host. The second model, evasion of the innate immune system, was tested by assessing the infectivity of the *ospC* mutant in mice deficient for myeloid differentiation protein 88 (MyD88). Recent studies have shown that *B. burgdorferi* is prevented from reaching high cell numbers in the mammalian host by MyD88-dependent signaling pathways. The *ospC* mutant was incapable of infecting MyD88-deficient mice, suggesting that the role of OspC cannot be related solely to evasion of MyD88-mediated innate immunity. These results reiterate the importance of OspC in mammalian infection and eliminate simple models of function for this enigmatic protein.

Spirochetes of the genus *Borrelia* are obligate parasites transmitted by arthropods, and many are pathogenic to humans. Relapsing fever spirochetes produce a recurring bacteremia in the blood, whereas Lyme disease spirochetes persist at low levels in various tissues, including skin, nervous system, heart, and joints (42). To survive for extended periods within the mammalian host, the borreliae have apparently evolved multiple mechanisms to evade the host immune system. Of the *Borrelia* proteins associated with immune evasion, all are plasmid-encoded surface components, although the molecular mechanisms differ among species and some have not been fully elucidated.

Borrelia burgdorferi, the causative agent of Lyme disease in the United States, can contain over 21 plasmids, several of which encode proteins that may assist in host immune evasion. The VlsE lipoprotein is encoded on linear plasmid 28-1 from an expression site that is preceded by >8 kb of silent, reiterated partial copies of *vlsE* (48). Each silent cassette varies from the others and can recombine with the *vlsE* expression site through a gene conversion-like mechanism, resulting in variation at the outer membrane. During mammalian infection, switching at the *vlsE* locus produces new outer surface variants that, in theory, are not recognized by previously produced antibodies (28, 49, 50). Other plasmid-encoded surface proteins of *B. burgdorferi* have been implicated in evading components of the innate immune system. The CRASP/Erp proteins bind the host complement-regulatory protein factor H and factor H-like protein-1/reconectin (19, 22, 23, 43). Binding of these host proteins by *B. burgdorferi* may prevent activation of the alternative pathway of complement.

Recently, our lab demonstrated that the major outer surface protein C (OspC) of *B. burgdorferi* is an essential virulence factor required for the initial stages of mammalian infection (18). Infectivity in immunocompetent mice was completely abrogated in a mutant *B. burgdorferi* strain lacking OspC. The mutant strain did not elicit an antibody response in the mice, nor could this strain infect severe combined immunodeficient (SCID) mice. Since SCID mice lack functional B and T cells, and the OspC mutant could not infect the SCID mice, the function of OspC does not seem to be resistance to the acquired immune system of the host. This suggested that OspC may play a role in avoiding clearance by the innate immune system or, alternatively, may fulfill a required physiological function during the initial infection of the mammal. Another *B. burgdorferi* plasmid-encoded protein, PncA, was previously shown to be required for spirochete survival in vivo but not during in vitro growth in Barbour-Stoenner-Kelly (BSK) medium (35). Similarly, the *ospC* mutant grows normally during in vitro cultivation but is unable to infect mice (18).

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Tilly and colleagues further demonstrated that OspC is required only at the initial stage of mammalian infection and is dispensable for persistence of *B. burgdorferi* in the mouse (44). The absolute and immediate requirement for OspC in the mammal explains why this protein is produced by *B. burgdorferi* within the feeding tick as the spirochete prepares for transmission to the mammalian host. Although our data do not support a requirement for OspC by *B. burgdorferi* in ticks (18, 44), other reports suggested that OspC may also be important for invasion of the tick salivary glands and binding of tick salivary proteins, indicating roles for OspC in both hosts (34, 37). However, the function of OspC within the mammalian host has not been determined.

Here, we report the results of experiments designed to distinguish between two simple models of OspC function in the mammalian host. The first model (physiological model) presumes that OspC provides a required physiological function during mammalian infection. The second model (innate immune model) proposes that OspC is necessary for *B. burgdorferi* to survive some aspect of the innate immune system. We tested these models by two methods. The first method assessed the ability of the *ospC* mutant to grow in dialysis membrane chambers (DMCs) implanted in the peritoneal cavities of rats. Growth of *B. burgdorferi* in DMCs was previously shown to require physiological functions, such as the nicotinamidase PncA, but not mechanisms of putative immune evasion, such as VlsE (35). The second method tested the ability of the *ospC* mutant to infect mice deficient in myeloid differentiation marker 88 (MyD88). MyD88 is a common adapter molecule required by most Toll-like receptors (TLRs) and other signaling pathways to trigger the innate immune response to microbial invasion. TLRs recognize common microbial components such as lipopolysaccharides, peptidoglycan, lipoproteins, and other pathogen-associated molecular compounds. Once activated by ligand binding, TLRs utilize signal transduction pathways (most of which include the common adapter MyD88) to produce inflammatory cytokines and other immune effectors. Several studies have demonstrated that TLRs and other MyD88-dependent pathways are significant mechanisms by which the host innate immune system limits *B. burgdorferi* proliferation (26, 31, 45, 46). In this study, we found that the OspC mutant was capable of growth and host adaptation within the largely immune-privileged DMCs, indicating that OspC does not fulfill a required physiological function in DMCs. However, further experiments indicated that OspC does not provide a survival mechanism for *B. burgdorferi* to evade the MyD88-mediated innate immunity. These results, combined with previous data, indicate a more complex function for OspC than predicted by our simple models: although required for the initial infection of mammals, OspC does not provide a singular role in survival of specific branches of the innate or acquired immune system so far investigated, nor does it provide a strictly physiological function for *B. burgdorferi*.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *B. burgdorferi* strains were grown at 35°C in liquid BSKII medium supplemented with 6% rabbit serum (Pel Freez Biologicals, Rogers, AR) or in solid BSK medium incubated at 35°C under 2.5% CO_2 (38). The *B. burgdorferi* type strain B31 (ATCC 35210) was originally isolated from a tick collected on Shelter Island, N.Y. (8). The genomic sequence of *B. burgdorferi* B31 culture MI has been determined (10, 15). B31 A3 is a low-passaged, transformable, infectious clonal derivative of B31 MI (14). The *ospC* mutant strain (*ospC7*) and the complemented strain (*ospC7*/*ospC*$^{+}$*4*) were constructed in B31 A3 and previously described by Grimm et al. (18). Briefly, allelic exchange was used to disrupt the *ospC* locus by insertion of a kanamycin resistance marker. The complemented strain was constructed by integrating a wild-type copy of *ospC* adjacent to the mutated allele. The plasmid contents of the mutant and complemented strains were identical to that of the wild-type parent strain, as confirmed by PCR analysis (14, 18, 36).

Bacterial growth in DMCs. Sterile Spectra/Por dialysis membrane tubing with a molecular mass cutoff of 8,000 Da (Spectrum Laboratories, Rancho Dominguez, CA) was filled with ~4 ml BSKII medium containing ~5×10^3 *B. burgdorferi* cells/ml and tied shut. The DMCs were then surgically implanted into the peritoneal cavities of anesthetized Sprague-Dawley rats (Harlan Sprague-Dawley Inc., Indianapolis, IN). Total genomic DNA was isolated from the unused portion of the *B. burgdorferi* inoculum, and plasmid content was determined by PCR analysis to confirm that all strains were isogenic (14). After 4 to 12 days, depending on the experimental time course, rats were euthanized and DMCs removed. Growth of DMC-cultured spirochetes was assessed by direct cell count using a Petroff-Hausser counting chamber, and host adaptation was determined by analysis of cell lysates by silver-stained gel and immunoblotting assays (18). Cell lysates used for two-dimensional gel analysis were obtained from DMCs inoculated at 1×10^7 cells/ml and removed after 72 h. Host adaptation was confirmed as described above.

Mouse infections. Host-adapted *B. burgdorferi* cells harvested from DMCs were inoculated into three RML mice/strain. RML mice are an outbred strain of Swiss Webster mice maintained at Rocky Mountain Laboratories since 1937. Mice were inoculated and infectivity assessed by seroconversion to *B. burgdorferi* antigens and reisolation of spirochetes as previously described (17). All animal experiments were performed in accordance with the guidelines of the National Institutes of Health. Animal protocols were approved by the institution's Animal Care and Use Committee. Rocky Mountain Laboratories is accredited by the International Association for Assessment and Accreditation of Laboratory Animal Care.

MyD88-deficient mice were provided by Shizuo Akira (1) and were maintained as heterozygous breeding pairs at the sixth generation backcross on the C57BL/6 background. Homozygous MyD88-deficient offspring were identified by PCR-based genotyping (5). C57BL/6 mice were obtained from the National Cancer Institute (Bethesda, MD). Mice were housed in ventilated cages in the Animal Resource Center at the University of Utah Medical Center (Salt Lake City, UT) according to the National Institutes of Health guidelines for care and use of laboratory animals.

PCR detection of *B. burgdorferi* DNA in mouse tissues. DNA was prepared from rear ankle, heart, and ear tissues at the time of sacrifice as previously described (46). Briefly, tissues were incubated for 5 h at 37°C in an 0.1% collagenase A (Roche, Indianapolis, IN) solution. An equal volume of 0.2-mg/ml proteinase K (Invitrogen Life Technologies, Carlsbad, CA) was added, and samples were incubated overnight at 55°C. DNA was recovered by phenol-chloroform extraction and ethanol precipitation. DNA concentration was determined by optical density at 260 nm. PCR detection of *B. burgdorferi* DNA in mouse tissues was conducted by continuous fluorescent monitoring PCR with the LightCycler 3.5 (Roche Molecular Biochemicals) using software from the manufacturer, as described previously (5, 31). The oligonucleotide primers used for amplification of the *B. burgdorferi recA* gene were nTM17.F (5′-GTGGATCTA TTGTATTAGATGAGGCTCTCG-3′) and nTM17.R (5′-GCCAAAGTTCTG CAACATTAACACCTAAAG-3′).

Two-dimensional gel electrophoresis and immunoblot analysis of DMC-cultivated cell lysates. For separation in the first dimension by nonequilibrium pH gradient gel electrophoresis (NEPHGE), spirochetes from DMCs were suspended in C_4TT NEPHGE buffer consisting of 7 M urea (Promega, Madison, WI), 2 M thiourea (Fisher Scientific, Pittsburgh, PA), 4.0% (wt/vol) CHAPS {3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate} (EMD Biosciences, La Jolla, CA), 1.0% (vol/vol) Triton X-100 (Bio-Rad Laboratories, Inc., Hercules, CA), 65 mM dithiothreitol, and 2.0% (vol/vol) preblended ampholytes pH 3 to 9.5 (Amersham Life Sciences, Piscataway, NJ) to a final cell density of 5×10^6 cells per μl. The samples were incubated overnight at 23°C with gentle agitation, clarified by ultracentrifugation (435,700 × *g*, 30 min, 23°C), and stored at −80°C until separation. Five microliters (2.5×10^7 cells) of each sample was loaded onto a 2.0-mm by 12-cm tube gel consisting of 8 M urea, 4.4% Duracryl acrylamide (Genomic Solutions, Ann Arbor, MI), 4.0% (wt/vol) CHAPS, 1.0% (vol/vol) Triton X-100, and 2.0% (vol/vol) preblended ampholytes pH 3 to 9.5. The first dimension was focused at 200 V for 1 h and then increased to 600 V for 4 h (a total of 2,600 V · h). The tube gels were extruded and stored at −80°C until

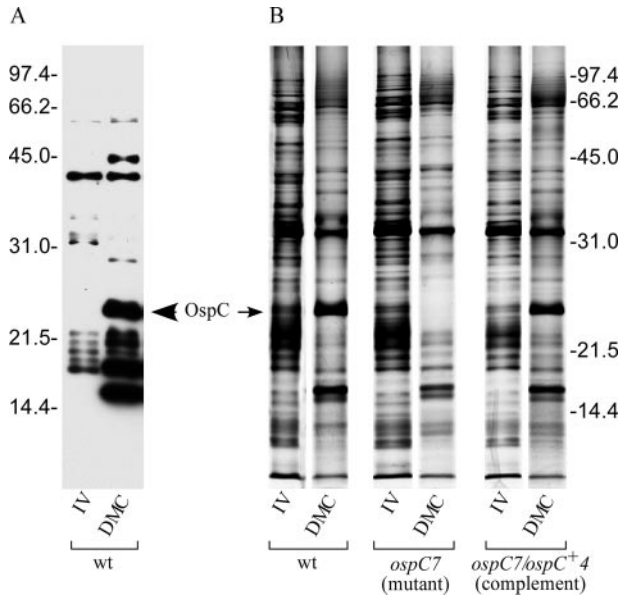

FIG. 1. Survival and host adaptation of *B. burgdorferi* strains in dialysis membrane chambers. (A) Cell lysates of in vitro (IV)- and DMC-grown B31 A3 (wild type) were immunoblotted and incubated with pooled sera from mice infected with *B. burgdorferi*. (B) Silver-stained SDS-polyacrylamide gel comparing *B. burgdorferi* strains grown in vitro (IV) to those grown in DMCs. Protein bands correlating to OspC and other lower-molecular-weight proteins are induced in DMC-cultured *B. burgdorferi* cells (except in the mutant strain, which lacks OspC). Molecular mass standards (in kilodaltons) are indicated on the outside of both panels.

separated in the second dimension by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Prior to the separation of proteins in the second dimension, focused NEPHGE tube gels were equilibrated twice (10 ml, 10 min, 23°C) with SDS equilibration buffer composed of 3 M urea, 2.0% (wt/vol) SDS (Fisher), 1.0% dithiothreitol, and 10% (vol/vol) glycerol in 125 mM Tris (pH 8.8). Standard SDS-PAGE was performed by laying the tube gel onto a 13.5-cm × 1.5-mm × 14-cm (length by width by height) 12.5% acrylamide gel on a Hoefer SE600 gel apparatus and running the apparatus at 35 mA per gel. Broad-range protein standards were used to estimate relative molecular masses (Bio-Rad, Hercules, CA). For two-dimensional immunoblotting, the proteins were electrophoretically transferred to nitrocellulose, stained, and blocked as described previously (9). Pooled mouse immune serum, as the primary antibody, was diluted 1:2,000 in Tris-buffered saline (150 mM NaCl in 10 mM Tris-HCl, pH 8.0) with the addition of 0.1% Tween 20 (TBS-T_{20}) and applied to the blot (1 h, 24°C). The blot was washed twice in 100 to 200 ml TBS-T_{20} for 10 min to remove residual primary antibody. Horseradish peroxidase-conjugated goat anti-mouse antibody (Sigma Chemical Co., St. Louis, Mo.) was diluted 1:5,000 in TBS-T_{20} and applied to the blot (45 min, 24°C), followed by three washes with 100 to 200 ml of TBS-T_{20}. Reactive bands were visualized with the Enhanced Chemiluminescence kit (Amersham) in accordance with the manufacturer's specifications.

RESULTS

Host adaptation of the *ospC* mutant strain. *B. burgdorferi* cells have been cultured and reisolated from chambers implanted within the peritoneal cavities of mice and rats (2, 21). These host-adapted *B. burgdorferi* cells display many characteristics indicative of adaptation to the mammalian host (2, 7). Among the changes that we observed from DMC-cultivated *B. burgdorferi* are the up-regulation of OspC and several unidentified, smaller proteins, readily visible on an immunoblot probed with mouse antisera against *B. burgdorferi* (Fig. 1A). Purser and coworkers demonstrated the utility of *B. burgdorferi* growth in DMCs to differentiate between spirochetal proteins that provide a required physiological function (such as PncA) and proteins that function in immune evasion (such as VlsE) (35). Previously, we showed that the OspC mutant could not infect SCID mice, suggesting an unanticipated physiological function for OspC (18). Therefore, we tested the *ospC* mutant, complement, and wild-type strains in DMCs to determine if OspC provided an essential physiological role (Table 1). Ten rats were used per strain, and each strain survived and grew to similar cell densities, although some variability among individuals within groups was observed. These results indicated that OspC did not appear to provide a typical physiological function required for growth in the DMC.

Although all three strains grew in the DMCs, it was not apparent whether the *ospC* mutant could alter its protein composition in adaptation to the mammalian environment. Therefore, total protein lysates of in vitro- and DMC-grown *B. burgdorferi* cells were visualized by silver staining an SDS-polyacrylamide gel separated in a single dimension (Fig. 1B). All strains cultivated in the DMCs, including the *ospC* mutant, displayed significantly different protein profiles from those of in vitro-grown cultures. Among the many protein changes observed was the induction of OspC in the wild-type and complemented strains (although OspC was absent from the mutant strain, as expected). Therefore, under DMC growth conditions, OspC does not appear to be required for sensing the host environment, nor does OspC seem to be involved in a signaling cascade to adapt to the mammalian environment.

Antigenic composition of DMC-cultivated spirochetes. Potentially, the loss of a major membrane protein such as OspC could result in compensatory changes in expression of other membrane proteins, either to physically compensate for the absent protein or if OspC interaction is required by other proteins to function. We therefore examined the proteome of the host-adapted *ospC* mutant in greater detail by two-dimensional gel and immunoblot analysis of total cell lysates (Fig. 2). Although some proteins showed minor variation (e.g., spots marked with asterisks, Fig. 2), the overall antigenic profiles of the three strains were similar, suggesting that OspC does not significantly affect expression of other antigenic proteins.

TABLE 1. *B. burgdorferi ospC* mutant grows and adapts in DMCs but remains noninfectious for mice

B. burgdorferi strain	DMC result:		Mouse infectivity by DMC-grown cells[c]
	Growth[a]	Host adaptation[b]	
WT[d]	+	+	3/3
ospC7 (mutant strain)	+	+	0/3
ospC7/*ospC*$^+$*4* (complemented strain)	+	+	3/3

[a] Growth within DMCs represents at least four doublings of *B. burgdorferi* from the initial inoculum, and all strains reached densities of $>10^6$ cells/ml.

[b] Host adaptation was assessed by SDS-PAGE and subsequent immunoblot analysis (Fig. 1).

[c] Number of mice infected/number of mice injected with DMC-grown *B. burgdorferi*. Mouse infectivity was assessed by serological response 4 weeks postinfection against the early antigen P39 (40) and total *B. burgdorferi* cell lysate.

[d] WT, wild type.

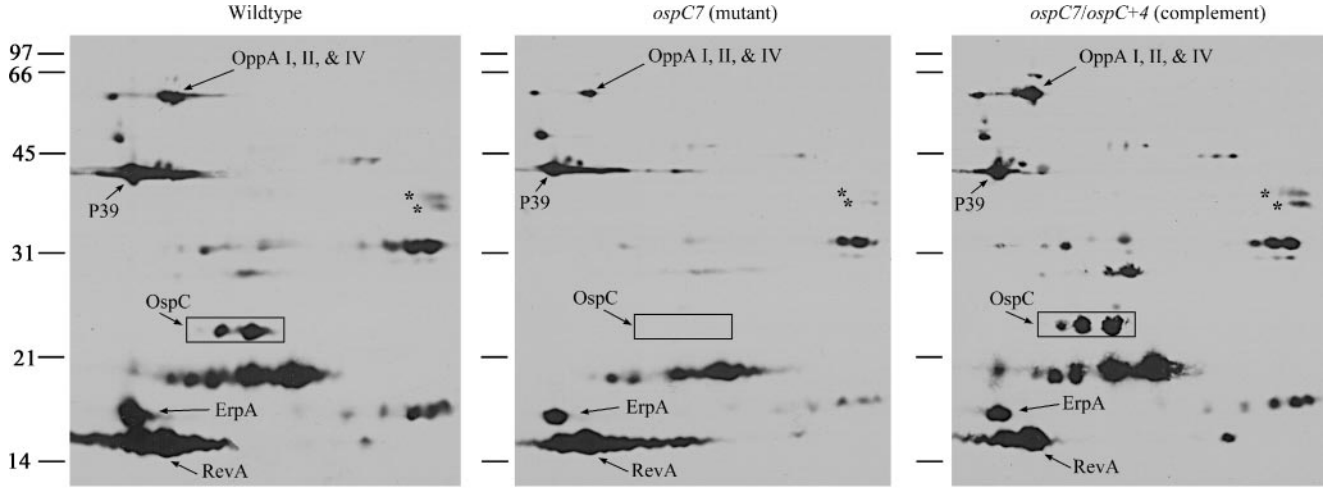

FIG. 2. Two-dimensional NEPHGE immunoblot of host-adapted *B. burgdorferi*. The immunoblot was incubated with pooled sera from mice infected with *B. burgdorferi*. Acid ends are to the left. Presumptive identities of OspC and other proteins are indicated based on the molecular weight and position in NEPHGE, relative to previously identified proteins (32). Asterisks denote proteins that may be at lower concentrations in the OspC mutant than in wild-type and complemented strains. Positions of molecular mass standards (in kilodaltons) are indicated. P39 has also been referred to as BmpA (41).

Host-adapted *ospC* mutant spirochetes cannot infect mice. Since the protein profiles of DMC-cultivated *ospC* mutant spirochetes resembled those of the wild type and complemented clones, we wondered whether mammalian infectivity might be restored in host-adapted *ospC* mutant cells. Infectivity was assessed by inoculating DMC-grown bacteria directly into mice and examining the serological response after 4 weeks. Sera from all mice inoculated with either wild-type A3 or the complemented strain contained antibodies that recognized *B. burgdorferi* proteins (Table 1). However, sera from mice inoculated with the DMC-grown *ospC* mutant strain did not exhibit a detectable antibody response toward *B. burgdorferi* (data not shown). Apparently, the induction of specific proteins following growth in DMCs does not restore infectivity to spirochetes lacking OspC.

***B. burgdorferi ospC* mutant does not infect MyD88$^{-/-}$ mice.** The ability of the *ospC* mutant to grow and host-adapt in the relatively immune-privileged DMC indicated that OspC does not fulfill a required physiological function but perhaps provides a means to evade the immune response of the mammal. Previous results for immunocompetent and SCID mice indicated that the adaptive immune response was not required to eliminate the *ospC* mutant, suggesting that OspC may allow *B. burgdorferi* to avoid clearance by some component of the innate immune system (18). Several studies have identified TLR-2 as the major receptor for *B. burgdorferi* lipoproteins and demonstrated that TLR-2 and MyD88-dependent signaling pathways prevent *B. burgdorferi* from attaining high cell numbers during infection (4–6, 20, 26, 46).

To determine if OspC is required to survive TLR-mediated killing or other MyD88-dependent pathways, we infected immunocompetent and MyD88-deficient mice with the *ospC* mutant, complement, and wild-type strains (Table 2). Although the wild-type and *ospC*-complemented strains infected both sets of mice, the *ospC* mutant strain did not infect either, indicating that elimination of TLR signaling does not obviate the requirement for OspC by *B. burgdorferi* in the mammal. Since the *ospC* mutant was unable to infect mice lacking components of either the acquired (18) or innate branches of the immune response, the essential role of OspC in mammalian infection is other than immune evasion or not solely restricted to resisting the single components of host immunity examined.

DISCUSSION

B. burgdorferi is transmitted to the mammalian host as *Ixodes* ticks feed. The blood meal of the tick appears to act as a trigger for *B. burgdorferi* to restructure its outer membrane in prepa-

TABLE 2. *B. burgdorferi ospC* mutant does not infect MyD88$^{-/-}$ mice

Mouse[a] and *B. burgdorferi* strain	Detection of *B. burgdorferi*	
	PCR[b]	Culture[c]
MyD88$^{+/+}$		
BSK[d] (negative control)	0/1	0/1
WT[e]	4/5	5/5
ospC7 (mutant)	0/5	0/5
ospC7/*ospC*$^{+}$*4* (complement)	3/5	2/5
MyD88$^{-/-}$		
BSK[d] (negative control)	0/2	0/2
WT	5/5	5/5
ospC7 (mutant)	0/5	0/5
ospC7/*ospC*$^{+}$*4* (complement)	4/5	5/5

[a] All mice derived from a C57BL/6 background as described in Materials and Methods.
[b] Number of PCR-positive mice/number of mice injected, assessed by PCR detection of the *B. burgdorferi recA* gene from mouse tissues (ankle, heart, and ear). Positive results were based on detection in at least one tissue.
[c] Number of mice culture positive for *B. burgdorferi*/number of mice injected, based on the culture of mouse urinary bladders in BSK medium. After 2 weeks, cultures were examined by dark-field microscopy for the presence of spirochetes.
[d] Negative-control mice were injected with sterile BSK medium.
[e] WT, wild type.

ration for infection of the mammalian host. Among the changes that occur prior to transmission to the host is the expression of OspC on the outer surface of the bacterium (39). OspC remains on the outer surface of *B. burgdorferi* during migration to the tick salivary glands and during the initial infection of the mammal (25, 30, 39), although exceptions to this model have been observed. Ohnishi and colleagues noted OspC-negative cells deposited in the host dermis tissue by feeding ticks, suggesting that OspC may not be required for migration from the tick midgut to the salivary glands or transmission (33). However, the timing of successful mouse infections correlated with the expression of OspC on the surface of *B. burgdorferi*. Grimm et al. constructed an *ospC*-minus mutant and demonstrated that whereas this strain could be detected in the salivary glands of feeding ticks, it was unable to infect mice by either tick bite or needle inoculation (18). Complementation with a wild-type copy of *ospC* restored infectivity, confirming the requirement for this gene product for mammalian infection. Additionally, the *ospC* mutant did not provoke an antibody response in immunocompetent mice, nor could it infect SCID mice. Therefore, to distinguish between the physiological model and the innate immune model of OspC function, we assessed the viability of the *ospC* mutant in DMCs implanted within rat peritoneal cavities. The molecular mass cutoff of 8,000 Da of the DMC excludes most components of the immune system from the chambers and therefore provides a relatively immune-privileged environment. These conditions allow some *B. burgdorferi* strains to grow when they normally would not persist in immunocompetent hosts (35). However, inflammation in murine DMC models has been shown to influence transcript levels and protein expression in DMC-cultivated *B. burgdorferi*, indicating that some components of the inflammatory response are capable of traversing the DMC (11). The OspC-deficient *B. burgdorferi* survived, grew, and adapted in the DMC similarly to the wild-type and complemented strains. This indicates that OspC is not providing an essential physiological function for the spirochete in the DMC environment. In contrast, PncA provides an essential function for *B. burgdorferi* and is required for survival of the spirochete in the DMC (35). However, it is possible that OspC is not essential for *B. burgdorferi* growth in DMC culture conditions but does provide a physiological function required to survive in the host outside the chambers.

Although OspC is not required for spirochete growth in DMCs, it was possible that OspC was involved in sensing and adapting to the mammalian host. The crystal structure of OspC displays a resemblance to the ligand-binding domain of the aspartate receptor of *Salmonella enterica* serovar Typhimurium, involved in chemotaxis (13, 24, 47). This observation suggested a potential role for OspC in detecting and/or signaling cues associated with the change from the tick to the mammal. However, the *ospC* mutant grew and host adapted similarly to the wild-type and complemented strains (Table 1 and Fig. 1), indicating that OspC is not necessary for sensing and adapting to aspects of the mammalian host environment mimicked in the DMC. These findings were supported by two-dimensional immunoblotting of *B. burgdorferi* cell lysates from DMC-grown bacteria in which the antigenic protein profiles of the wild-type, OspC mutant, and complemented strains did not differ substantially from one another (Fig. 2). These data also indicate that other immunogenic proteins (i.e., RevA and ErpA) do not appear to be altered in abundance to compensate for the lack of OspC, even in the mammalian-adapted spirochetes grown in the DMCs.

Further, the host-adapted *ospC* mutant strain could not infect immunocompetent mice, indicating that other proteins induced during growth in DMCs could not fulfill the function of OspC. Although *B. burgdorferi* lacks any sequence paralogs of *ospC*, it is possible that functional homologs existed in the genome. The sequential expression of potential OspC substitutes might explain why OspC is required only during the initial stage of infection but is not necessary for persistence of the spirochete (44). However, if functional homologs of OspC are present, they apparently are not expressed in the DMC.

Since the *ospC* mutant strain cannot infect immunodeficient SCID mice or elicit an antibody response in immunocompetent mice but can survive in DMCs, we hypothesized that OspC may be required for evading some component of the host innate immune response. TLRs play a major role in the initial host response to microbial infections, and TLR-2 was specifically shown to respond to the lipoproteins of *B. burgdorferi* and aid in controlling spirochete numbers (6, 26, 46). Many of the Toll-like receptor family members signal through the adapter protein MyD88 (see reference 3 for a recent review). However, other MyD88-dependent pathways, in addition to the TLR-2 pathway, play important roles in limiting *B. burgdorferi* proliferation in host tissues (5, 26). The MyD88 signaling pathway is known to be important in several aspects of innate host defense including activation of the phagocyte-dependent killing pathways that include induction of reactive oxygen intermediates and nitric oxide production and up-regulation of the production of defensins. Although wild-type and complemented strains could establish infections in MyD88$^{-/-}$ mice, the *ospC* mutant strain could not (Table 2). Therefore, the sole function of OspC is not evasion of these and other MyD88-dependent pathways of host defense.

The crystal structure of OspC also displays an overall three-dimensional similarity to that of the variant surface glycoprotein (VSG) of *Trypanosoma brucei*, the causative agent of African sleeping sickness (13). VSG undergoes antigenic variation and likely protects key membrane proteins from attack by the host immune system by masking the cell in a thick, shield-like barrier (reviewed in reference 12). Although the function of OspC may not be identical to that of the VSGs of *T. brucei*, we have shown that the *ospC* mutant can grow in the largely immune-privileged DMC but cannot infect mice deficient in components of acquired or innate immunity. The structural similarity to VSG of *T. brucei* supports the role of OspC in survival of some aspect of the host immune system, perhaps shielding the *B. burgdorferi* outer membrane from attack by the innate immune system or in binding a component of that system. *B. burgdorferi* is susceptible to killing by other branches of the innate immune system that are not altered in MyD88$^{-/-}$ mice. Polymorphonuclear leukocytes, among the first immune cells to respond to microbial invasion in dermis tissues, have been shown to possess microbicidal activity against *B. burgdorferi* and suggest an area for further investigation (16, 27, 29). Alternatively, OspC may provide protection from other components of host immunity, such as complement. Finally, it is possible that OspC may provide multiple functions

essential for survival in the mammalian host, including evasion of both the acquired and innate branches of the immune system.

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