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# Comprehensive seroprofiling of sixteen *B. burgdorferi* OspC: Implications for Lyme disease diagnostics design

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**Abstract** Early diagnosis of Lyme disease (LD) is critical to successful treatment. However, current serodiagnostic tests do not reliably detect antibodies during early infection. OspC induces a potent early immune response and is also one of the most diverse proteins in the *Borrelia* proteome. Yet, at least 70% of the amino acid sequence is conserved among all 21 known OspC types. We performed a series of comprehensive seroprofiling studies to select the OspC types that have the most cross-reactive immunodominant epitopes. We found that proteins belonging to seven OspC types detect antibodies from all three infected host species regardless of the OspC genotype of the infecting strain. Although no one OspC type identifies all seropositive human samples, combinations of as few as two OspC proteins identified all patients that had anti-OspC antibodies.


## Introduction

Lyme disease (LD), caused by the spirochete *Borrelia burgdorferi*, is the most prevalent vector-borne disease in the northern hemisphere. Early diagnosis is critical to successful treatment and complete recovery [1,2]. However, clinical and serological diagnosis of Lyme disease is particularly difficult due to the phenotypic heterogeneity within and among species of the spirochete [3,1]. Even in regions where only one *B. burgdorferi* species is found, Lyme disease progresses very differently from one patient to another [4].

Current serodiagnostic tests for Lyme disease lack sensitivity and affinity for detection of anti-*B. burgdorferi* antibodies in the early stages of the disease. Sensitivity seldom exceeds 50% [5–8]. OspC was first identified as a seroreactive major outer surface protein in a subset of *B. burgdorferi* strains [9,10]. It is a virulence factor upregulated just prior transmission to the mammalian host and is indispensable for establishing infection [11–14]. Furthermore, OspC is the major protein expressed on the surface of *B. burgdorferi* during the first stages of infection [15] and induces a strong IgM immune response early on [16].

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Therefore, it is an essential antigen to include in serodiagnostic assays for early Lyme disease [17–23].

OspC is also one of the most diverse and heavily studied proteins in the *Borrelia* proteome. Distinct *ospC* genotypes are correlated with niche preference in natural reservoir species and invasiveness, pathogenesis and clinical manifestations in humans [24–31]. Twenty-one known OspC phyletic groups (referred to as OspC genotypes) classified by letters A to U [32–34] are distinguished by at least 8% amino acid sequence divergence. Given that there is at least 70% homology between all OspC genotypes [33], the presence of common epitopes that can be targeted for the development of new immunoprophylatic components has been explored [35]. We performed a series of comprehensive seroprofiling studies using serum panels from naturally infected white-footed mice, dogs and humans to screen for the OspC types that have common or cross-reactive immunodominant epitopes.

## Materials and methods

### *B. burgdorferi* strains

*B. burgdorferi* isolates were cultured from blood or *erythema migrans* skin biopsies of human patients seen at the Westchester Medical Center (kindly provided by Dr. Gary Wormser, New York Medical College (NYMC), Valhalla, NY). Fifteen OspC group-specific *B. burgdorferi* human isolates were typed for OspC phyletic group in Dr. Ira Schwartz laboratory (NYMC, Valhalla, NY) and were kindly provided to us for this study. Low passage *B. burgdorferi* were grown at 34 °C in Barbour-Stoenner-Kelly H (BSK-H) medium supplemented with antibiotic mixture for *Borrelia* (Sigma-Aldrich, St. Louis, MO). Total DNA was isolated from spirochetes using IsoQuik Nucleic Acid Extraction Kit (ORCA Research Inc., Bothell, WA). Patients provided informed consent and experimentation guidelines were followed as approved by the New York Medical College IRB.

### Infection of mice with *B. burgdorferi*

Viability and number of spirochetes grown to mid- or late-log phase was done by dark field microscopy (Axio Imager, Zeiss, Germany). $10^5$ bacteria were used to infect C3H-HeJ mice subcutaneously. Three weeks later mice were bled and the serum was tested for the presence of *B. burgdorferi* antibodies using the ViraBlot test (VIRAMED Biotech AG). Animal experimentation guidelines were approved by UTHSC's Animal Care and Use Committee.

### Serum panels from naturally infected hosts

For the purpose of seroprofiling we used serologically characterized serum panels only. A panel, $n=43$, was obtained from the natural reservoir of *B. burgdorferi*, the white-footed mouse (*P. leucopus*) and was previously screened for *B. burgdorferi* infection by C6 ELISA (Immunetics, Boston, MA). A panel, $n=38$, was obtained from naturally infected dogs with Lyme disease previously tested for *B. burgdorferi* infection by whole cell sonicate ELISA. A panel, $n=25$, was obtained from naturally infected humans with Lyme disease from the United States. This panel was obtained from patients presenting with *erythema migrans* and was previously screened for *B. burgdorferi* infection by C6 ELISA (Immunetics, Boston, MA). The last panel, $n=40$, was obtained from naturally infected humans with Lyme disease from Europe. This panel comprises serum from 19 patients presenting with *erythema migrans* with IgM and IgG antibodies to *B. burgdorferi*; 11 patients with IgM and IgG antibodies to *B. burgdorferi* and 10 patients with IgM antibodies to *B. burgdorferi*. These 21 patients did not present with *erythema migrans*. Patients provided informed consent and experimentation guidelines were followed.

### Cloning, expression and purification of recombinant OspC proteins

A 560 bp-fragment of each *B. burgdorferi* ospC type gene was amplified by PCR. A Nde I/BamH I fragment was cloned into pET9c (Novagen, Gibbstown, NJ). Plasmids were sequenced (GENEWIZ, Inc., South Plainfield, NJ) and the sequences of *ospC*-fragments were confirmed by ClustalW alignment with Genbank published sequences. Recombinant OspC proteins were expressed in *Escherichia coli* BL21 (DE3) and purified by ion exchange chromatography using Q-Sepharose Fast Flow (GE Healthcare, Sweden). Protein concentration was determined with the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA). OspC proteins were analyzed on a 15% SDS-PAGE Coomassie stained gel.

### OspC seroprofiling

OspC-immunoarrays were done using ELISA. Purified recombinant OspC protein was used to coat Nunc MaxiSorp™ flat-bottom ELISA plates (eBioscience, San Diego, CA) and indirect ELISA was performed using serum (1:100) from C3H mice, *P. leucopus*, dog, or human. Species-specific IgG secondary antibody was used for mouse, *P. leucopus* and dog (1:50,000, Jackson ImmunoResearch, West Grove, PA). For human, anti-human IgM+IgG horseradish peroxidase-conjugated secondary antibody was used (1:50,000, Jackson ImmunoResearch, West Grove, PA).

## Results

### Cloning, expression and purification of group-specific OspC

Sixteen of the 17*ospC* genotypes endemic to the US were cloned. The *ospC* gene from 15 of the 17 genotypes were cloned from *B. burgdorferi* isolates cultured from blood or *erythema migrans* skin biopsies of human patients seen at the Westchester Medical Center (Valhalla, NY). These isolates were typed for OspC phyletic group by reverse line blotting in Dr. Ira Schwartz laboratory (NYMC) [36]. OspC genotype L was amplified from a plasmid constructed from *B. burgdorferi* DNA isolated from ticks. OspC genotype O is rare in the northeastern US and was not available. All *ospC* genes were cloned in an expression vector (pET9c) and sequences confirmed by ClustalW alignment against Genbank standards [33,26]. Each of the 16 recombinant OspC proteins (A–N, T and U) was expressed in *E. coli* BL21(DE3)pLys devoid