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Humoral Immune Response to Outer Surface Protein C of *Borrelia burgdorferi* in Lyme Disease: Role of the Immunoglobulin M Response in the Serodiagnosis of Early Infection

BRENDA P. FUNG, GAIL L. McHUGH, JOHN M. LEONG, AND ALLEN C. STEERE*

Division of Rheumatology/Immunology, New England Medical Center, and Tufts University School of Medicine, Boston, Massachusetts 02111

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We determined the humoral immune response to outer surface protein C (OspC) of *Borrelia burgdorferi* in patients with early or late manifestations of Lyme disease and investigated the use of this antigen in the serodiagnosis of early infection. The *ospC* gene from the low-passage human isolate 297, a North American *B. burgdorferi* strain, was used to make a recombinant maltose-binding protein (MBP)-OspC fusion protein for serologic tests. This gene showed 84 to 85% nucleotide sequence identity and 76 to 79% amino acid identity with *ospC* of *B. burgdorferi* B31 and 2591. The antibody responses to MBP-OspC were determined in serial sera from 15 patients with Lyme disease who were monitored for 4 to 12 years of illness, in single-serum samples from 189 patients with early or late manifestations of the disorder, and in serum samples from 106 control patients. Early in the infection, patients with erythema migrans or meningitis commonly had weak to strong immunoglobulin M (IgM) responses to OspC and sometimes weak to moderate IgG responses. Months to years later, weak to strong IgG reactivity with this protein was often apparent in patients with arthritis, but this response was weak or absent in patients with chronic neuroborreliosis. When acute- and convalescent-phase serum samples from patients with erythema migrans were tested for reactivity against MBP-OspC, the sensitivity of the IgM test was 73% and the specificity was 98%, with either enzyme-linked immunosorbent assay (ELISA) or Western blotting. We conclude that the majority of patients with Lyme disease have a prominent IgM response to OspC early in the illness, which is often followed by a prominent IgG response in patients with arthritis. For the serodiagnosis of early infection, the sensitivity and specificity of IgM ELISA and Western blotting were comparable or slightly improved when MBP-OspC was used as the antigen compared with tests in which spirochetal lysates were used.

Lyme disease, which is caused by tick-borne spirochetes of the *Borrelia burgdorferi* sensu latu complex, is endemic in North America, Europe, and Asia (4, 36). Three taxonomic groups of *B. burgdorferi* sensu latu have been described (2, 5, 23). To date, all North American isolates have been identified as *B. burgdorferi* sensu stricto (group 1), whereas most European and Asian isolates have been identified as *B. garinii* (group 2) or *B. afzelii* (group 3) (2, 5). In the United States, the illness often begins with a characteristic skin lesion, erythema migrans (37), followed by meningitis or facial palsy (28), intermittent episodes of arthritis (40), or a subtle encephalopathy or polyneuropathy (19). Diagnosis is usually based on the recognition of a characteristic clinical picture accompanied by an elevated antibody response to *B. burgdorferi* (36). However, serologic confirmation of the diagnosis has been complicated by the delay in the development of the humoral immune response to the spirochete (7, 34), by the cross-reactivity of certain spirochetal polypeptides with other antigens (10, 20, 21), and by the lack of standardization among laboratories (1, 13). The low sensitivity of serologic tests early in the infection has been a particular problem (10, 34).

The humoral immune response in patients with Lyme disease develops gradually over a period of months to years to more than 10 polypeptides of the Lyme disease spirochete (3, 7, 10, 16). Initial studies emphasized that the first response in patients with erythema migrans was often to the 41-kDa flagellar antigen of the spirochete (3, 7), but as many as half of control subjects had reactivity with this protein (10). Reactivity with a 39-kDa protein (p39) seemed to be specific for *B. burgdorferi* infection (35), but in patients with erythema migrans this response may be difficult to distinguish from p41 on Western blots when spirochetal lysates are used as the antigen (10). Likewise, reactivity with two of the major outer surface proteins, OspA and OspB, appeared to be specific for this infection, but the immunoglobulin G (IgG) responses to these proteins did not develop until late in the illness, if at all (7, 16). In Europe, only a small percentage of patients were found to have antibody reactivity with OspA and none responded to OspB (9, 41).

Wilske et al. reported that the most common early antibody response in European Lyme borreliosis was to a 22- to 23-kDa lipoprotein (41, 43), now called outer surface protein C (OspC) (42). In an initial study, this protein was expressed in 45% of European strains but rarely in North American strains, as determined by Western blotting (43). These investigators cloned and sequenced the *ospC* gene from *B. afzelii* PKo (12). Subsequently, the 26- or 27-kb circular plasmid carrying OspC was found to be carried by all isolates tested, including representative isolates of *B. burgdorferi*, *B. garinii*, and *B. afzelii*. However, the protein was not always expressed in cultured spirochetes (24, 29, 31, 42).

* Corresponding author. Mailing address: New England Medical Center, Division of Rheumatology/Immunology, NEMC no. 406, 750 Washington St., Boston, MA 02111. Phone: (617) 956-5789. Fax: (617) 956-4252.

As with the European experience, a recent analysis of the antibody responses to *B. burgdorferi* in American patients with Lyme disease, using spirochetal lysates of strain G39/40 as the antigen, indicated that the most prominent response in patients with erythema migrans was to a polypeptide in the 21-kDa region (10). An antigen of equivalent size reacted with monoclonal antibody L22 1F8, which was generated against the OspC protein of *B. afzelii* PKo (12). Thus, in both Europe and America, the OspC protein appears to be a good candidate antigen for early serodiagnosis.

The purpose of the current study was to describe the humoral immune response to a recombinant OspC in North American patients with early or late manifestations of Lyme disease and to assess the potential role of this protein in the serodiagnosis of early infection.

MATERIALS AND METHODS

Isolates of *B. burgdorferi* and antigen preparation. The standard strain of *B. burgdorferi* used in our laboratory for antigen preparations is a high-passage tick isolate, called G39/40, from Guilford, Conn. (38). An additional *B. burgdorferi* strain, called 297, which was isolated from the cerebrospinal fluid of a patient infected in Westchester County, N.Y. (38), was frozen in multiple aliquots at low passage; an antigen preparation was made from passage 10. During the course of this study, *B. burgdorferi* was recovered from erythema migrans skin lesions (38) from four patients from Lyme, Conn. These low-passage isolates, called LP3 (Lyme patient 3), LP4, LP5, and LP7, were found by Richard Marconi, Rocky Mountain Laboratories, Hamilton, Mont., to be *B. burgdorferi* strains on the basis of 16S rRNA typing. Antigen preparations were made from these four isolates after two to five passages. For each antigen preparation, spirochetes were grown to late-logarithmic phase in 1 liter of BSK-H medium (Sigma, St. Louis, Mo.) supplemented with heat-inactivated rabbit serum (GIBCO, Gaithersburg, Md.), pelleted by centrifugation at 12,000 × *g* for 20 min, washed twice in phosphate-buffered saline (PBS) containing 1 mM $MgCl_2$, and disrupted on ice with 12 30-s pulses of a cell sonicator (model 200; Branson Sonic Power Co., Danbury, Conn.). After centrifugation as above, the supernatants were snap-frozen and stored in multiple aliquots at −70°C.

Production of recombinant OspC. DNA from *B. burgdorferi* 297, which had been cloned at low passage (p10) by limiting dilution, was used as the template in PCR. We chose to use strain 297 because it was a low-passage *B. burgdorferi* human isolate. Published 5′ and 3′ sequences of *ospC* from the European *B. afzelii* PKo (12) were used to generate primers for PCR amplification of the nucleotide sequence corresponding to amino acids 21 to 212 of the OspC protein. The sense primer was 5′-CGTCTAGAAATTCAGGGAAAGGTGGG-3′, and the antisense primer was 5′-GCCTGCAGGATCTTATTA AGGTTTTTTTGGACT-3′; the underlined portions are restriction enzyme sites for *Xba*I and *Pst*I, respectively. The amplification was performed in a reaction volume of 100 μl containing 50 pmol of each primer, 200 μM deoxynucleoside triphosphates, 20 mM Tris-HCl (pH 8.2), 10 mM KCl, 6 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.1% Triton X-100, 10 ng of bovine serum albumin per μl, 100 ng of template DNA, and 2.5 U of *Pfu* DNA polymerase from *Pyrococcus furiosus* (Stratagene, La Jolla, Calif.). The reaction conditions were denaturation at 94°C for 1 min, annealing at 47°C for 2 min, and extension at 72°C for 1 min for 25 cycles. After cleavage with *Xba*I and *Pst*I, the PCR product was inserted at the 3′ end of the *malE* gene of the vector pMal-cRI (New England Biolabs, Beverly, Mass.) to generate a translational fusion product consisting of an OspC, lacking the putative leader sequence, linked to the C terminus of the *Escherichia coli* maltose-binding protein (MBP). The identity of positive clones was confirmed by nucleotide sequencing in both orientations by using the dideoxynucleotide-chain termination method (Sequenase version 2.0; U.S. Biochemical Corp., Cleveland, Ohio) and by reactivity with a monoclonal antibody to OspC (L22 1F8; a generous gift of B. Wilske, Max von Pettenkofer Institut, Munich, Germany).

The plasmid containing the *ospC* sequence was transformed into *E. coli* SR2 (18), and protein production was induced during logarithmic growth with 0.3 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 3 h at 30°C. Bacteria were washed in 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES, pH 8.0), resuspended in 10 mM HEPES (pH 8.0) containing 150 μg of phenylmethylsulfonyl fluoride per ml and 0.03 U of aprotinin per ml, and lysed by two or three passes through a French pressure cell at 14,000 lb/in^2. A cleared supernatant was collected after two consecutive centrifugations at 139,000 × *g* for 30 min at 4°C. MBP fusion proteins were purified on a cross-linked amylose column (22). After extensive washing, MBP fusion proteins (MBP-OspC) were eluted with column buffer (10 mM HEPES [pH 7.3], 200 mM NaCl, 1 mM EDTA) containing 10 mM maltose, and fractions were assayed for protein content and integrity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot. Coomassie blue-stained gels showed a single band representing MBP-OspC with an apparent molecular mass of 64 kDa, which reacted with monoclonal antibody L22 1F8. The protein concentration was determined by the Bradford assay (Bio-Rad Laboratories, Richmond, Calif.). Fusion proteins were used without further modification in enzyme-linked immunosorbent assays (ELISAs) and Western blots.

Patients. To determine the natural history of the antibody response to OspC, we tested serial serum samples, representing a period from disease onset through 4 to 12 years of illness, from 15 patients who had been cared for in the 1970s before the role of antibiotic therapy in this illness was known. All 15 patients had arthritis during the course of the illness. At the time of examination, joint swelling was rated as severe (score of 4), marked (score of 3), moderate (score of 2), or mild (score of 1).

In addition, our serum bank contains samples stored at −70°C from more than 1,400 patients with Lyme disease seen in our Lyme disease clinic during the past 17 years. For this study, we selected, in alphabetical order, acute- and convalescent-phase serum samples from 75 patients with erythema migrans and single samples from 40 patients with meningitis or facial palsy, 49 patients with arthritis, and 25 patients with encephalopathy or polyneuropathy. Samples from the first 25 patients in each group had been tested previously with sonicated spirochetal lysates as the antigen; reactivity to a 21-kDa polypeptide was demonstrated in 60% of the patients with erythema migrans (10). Patients with erythema migrans were classified as having localized skin infection or disseminated infection according to clinical criteria (26). For comparison, we tested serum samples from 106 patients who had recently had influenza vaccinations (an acute infectious disease antigen), multiple sclerosis or amyotrophic lateral sclerosis (other neurological syndromes), rheumatoid arthritis or systemic lupus erythematosus (other rheumatologic diseases), chronic fatigue syndrome (a chronic illness), or syphilis (another spirochetal infection) and from 28 normal control subjects.

ELISA. The IgM and IgG antibody responses to MBP-OspC

and to MBP alone were determined by indirect ELISA, as previously described (10). For all determinations, 96-well microtitration plates were coated with 5 μg of MBP-OspC per ml or 2.8 μg of MBP alone per ml (200 μl per well) and held overnight at 4°C. After being washed three times with 0.05% Tween 20 in PBS and again between each reaction step, the plates were blocked with 5% nonfat dried milk in PBS-Tween 20 (M-PBS) and incubated with 200 μl of patient sera diluted 1:100 and then with alkaline phosphatase-conjugated goat anti-human IgM (1:250 in M-PBS) or IgG (1:750 in M-PBS; TAGO, Burlingame, Calif.) for 45 min each at 37°C. The plates were then washed with PBS without Tween 20, and alkaline buffer containing 1 mg of *p*-nitrophenylphosphate (pNpp) per ml and 25 μM $ZnCl_2$ was added at room temperature. The reaction was stopped when the positive control reached an optical density reading (405 nm) of 1.0. An optical density for IgM above 0.03 and for IgG above 0.05 was considered positive; these absorbance values were 5 standard deviations (IgM) or 3 standard deviations (IgG) above the mean optical density readings of 28 normal control samples. A total of 8 of the 28 normal control samples, which were representative of the range of responses, and 1 positive control sample were included on each test plate.

Western blotting. SDS-PAGE and protein transfer were performed by using a miniblot system (Mini-Protean II; Bio-Rad), as previously described (10). For tests of patient sera, MBP-OspC and MBP alone (7.5 μg of each) or antigens from sonicated whole spirochetes (50 μg) were electrophoresed on a 10% polyacrylamide gel at 20°C and 175 V. The proteins were transferred to nitrocellulose paper (Bio-Rad) at 4°C and 100 V for 1 h. The paper was placed in Tris-buffered saline (TBS; 20 mM Tris, 500 mM NaCl [pH 7.6])–0.1% Tween 20 for 10 min and then cut into 2- or 4-mm strips. After being washed three times with 0.1% Tween 20 in TBS, and again between each reaction step, the strips were blocked in 5% nonfat dried milk in TBS–0.1% Tween 20 (M-TBS) and incubated with patient sera (1:250 in M-TBS) or monoclonal antibody to OspC (1:50 in M-TBS) and then with alkaline phosphatase-conjugated goat antibody to human IgM or IgG (1:3,000 in M-TBS; TAGO) or mouse Ig (1:3,000; A0162; Sigma) each for 1 h at room temperature. Bound antibody was detected by incubation at room temperature with substrate consisting of 367 μM nitroblue tetrazolium chloride and 345 μM 5-bromo-4-chloro-3-indolylphosphate in carbonate buffer (100 mM $NaHCO_3$, 1 mM $MgCl_2$ [pH 9.8]). The same positive and negative control serum samples were included with each set of blots.

Preadsorption of patient sera. Serum samples that showed reactivity to pure MBP in ELISA or Western blot were retested before and after preadsorption with MBP. A small piece of nitrocellulose was spotted with 2 to 5 μg of MBP, air dried, and blocked as above in M-TBS. For preadsorption, a portion of each serum sample (1:100 in M-TBS) was incubated with the nitrocellulose, for 3 h at room temperature or overnight at 4°C, with agitation. A control serum sample containing a high level of IgG against MBP was effectively depleted of MBP reactivity by this method.

Nucleotide sequence accession numbers. A partial nucleotide sequence of the *ospC* gene from *B. burgdorferi* 297 has been assigned GenBank accession number U08284. The sequences corresponding to OspC of strains B31, 2591, PBi, and PKo have been published (12, 14, 29) and are available through GenBank as accession numbers X69596, U01892, X69595 and X62162, respectively.

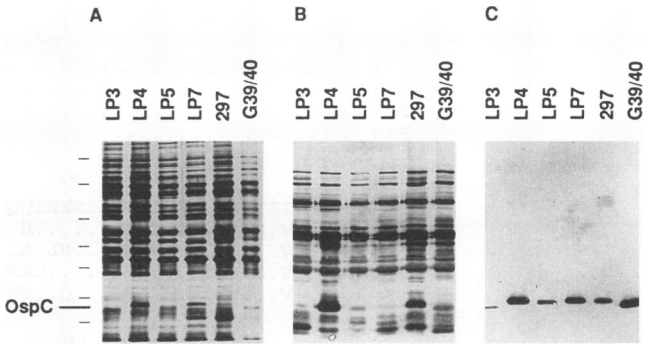

FIG. 1. Expression of OspC in six North American isolates of *B. burgdorferi*. (A) Total proteins (4 μg per lane) were separated by SDS-PAGE and silver stained (27). Molecular size markers are indicated at the left (from the top, 97.4, 66, 45, 36, 29, 24, and 20.1 kDa), and the location of OspC (22 kDa) is shown. (B) IgG Western blot of the same proteins reacted with serum from a North American Lyme disease patient with a high level of IgG reactivity to OspC. (C) An identical Western blot probed with monoclonal antibody L22 1F8 against OspC.

RESULTS

Expression of OspC in group 1 strains. It has been reported that North American *B. burgdorferi* strains inconsistently express a 21- to 23-kDa outer surface protein C (OspC) (24, 41, 43), which may explain the absence of reactivity to an antigen in this region in initial reports (7, 43). In this study, we examined antigen preparations from several different strains of *B. burgdorferi* for the presence of OspC: five low-passage human isolates from cerebrospinal fluid (CSF) (strain 297) or skin (LP3, LP4, LP5, and LP7), and one high-passage tick isolate (G39/40) for which 25 antigen preparations were available, covering a period of 117 serial passages. As determined by Western blotting with monoclonal antibody L22 1F8, OspC was expressed in the antigen preparations from all six isolates, although the reactivity was variable (Fig. 1). The apparent molecular mass of OspC in isolates G39/40 and LP3 was slightly lower (21 kDa) than that in isolates 297, LP4, LP5, and LP7 (22 kDa). However, the apparent molecular mass of OspC was the same in all 25 antigen preparations of G39/40, and the amount of antigen expressed did not appear to diminish upon extensive in vitro cultivation (data not shown).

Sequence of OspC from strain 297. Our next goal was to produce a recombinant OspC protein from a North American strain of *B. burgdorferi* for use in serodiagnostic tests of North American Lyme disease. PCR amplification was used to clone an *ospC* sequence from North American strain 297, with primers based on the DNA sequence of the *ospC* gene from *B. afzelii* PKo (12). The PCR product lacked sequences encoding the putative leader peptide. DNA sequence analysis of this cloned segment demonstrated 84 to 85% identity between the *ospC* genes of isolates 297, B31 and 2591, all of which are North American *B. burgdorferi* strains (14, 29). A similar level of DNA homology (79 to 84%) was found between any of these three *B. burgdorferi* strains and representative isolates of *B. garinii* and *B. afzelii* (12, 14). At the amino acid level, the OspC sequences of the three American strains had 76 or 79% identity with each other and 66 to 75% identity with strains from the other two groups. Thus, the degree of *ospC* sequence homology was not much greater within a species than between them. Amino acid differences between the five strains were scattered throughout the peptide sequence, although two

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                10        20        30        40        50        60
297                      NSGKGGN-TSANSADESVKGPNLTEISKKITESNAVVLAV
B31     MKKNTLSAILMTLFLFISCN....D..-.....................D....L...
2591    .......................D..-.....................................
PBi     ......................--..DSA.T.P-...A......V......D...FL....
PKo     ......................DSA.T.P....A............D...F....

                70        80        90       100       110       120
297     KEIETLLASIDELATKAIGKKIQQNGGLAVEAGHNGTLLAGAYTISKLITQKLDGLKNSE
B31     ..V.A..S....I.A.......H..N..DT.NN...S......A..T..K........-.
2591    ..V.........V.K....NL.A..-..NAG.NQ..S......V..T..AE.........
PBi     ..V.A..S.....S-.......KND.T.DN..NR.ES.I....E.........SV.-...
PKo     ..V...VL......K....Q..DN.N...ALNNQ..S......A..T...E..SK...L.

                130       140       150       160       170       180
297     KLKEKIENAKKCSEDFTKKLEGEHAQLGIEN--VTDENAKKAILITDAAKDKGAAELEKL
B31     G.....DA......T..N..KEK.TD..K.G--...AD..E...K.NGT.T...E..G..
2591    E......D....NKA..D..KSS..E...A.GAA..A...A...K.NGT.....Q.....
PBi     E.....KE..D..QK..T..KDS..E...QS--.Q.D.......K.HGT.....K...E.
PKo     E..TE.AK......E..N..KSG..D..KQD--A..DH..A...K.H.TT....K.FKD.

                190       200       210
297     FKAVENLAKAAKEMLANSVKELTSPIVAESPKKP
B31     .ES..V.S.................V........
2591    .ES.K..S...Q.T.N.........V...N....
PBi     ..SL.S.S...QAA.T.......N.V........
PKo     .ES..G.L...QVA.T.........V........
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FIG. 2. Deduced amino acid sequences of OspC from various strains. OspC amino acid sequences from strains 297, B31, 2591, PBi, and PKo were deduced from the corresponding nucleotide sequences (for GenBank accession numbers, see Materials and Methods) with the aid of the Genetics Computer Group software package (8). The alignments include spaces to maximize homology. Amino acid differences are shown; dots indicate identity with the 297 sequence, and dashes represent gaps. Underlined residues at the termini of the 297 sequence were encoded by the PCR primers used and do not represent the sequence of 297 *ospC*.

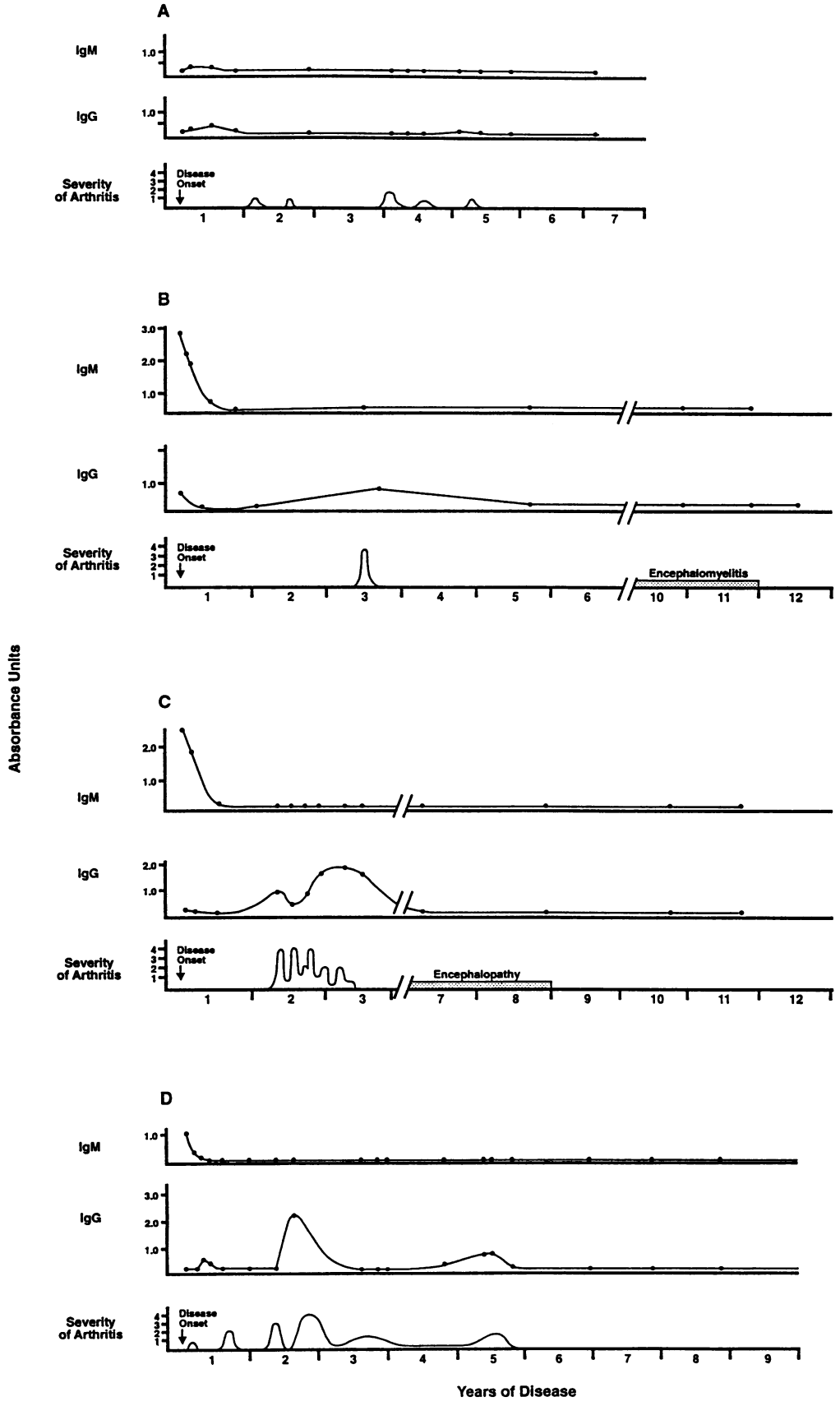
regions centered around residues 90 and 150 seemed to account for much of the diversity (Fig. 2).

Recombinant OspC in serodiagnostic tests. To obtain an easily purified recombinant protein for use in serologic assays, we used the *ospC* gene segment derived from strain 297 to generate MBP-OspC. We used purified MBP fusion proteins previously to analyze the immune response to OspA and OspB (16) and found that very few patient sera contained antibodies that recognized MBP. Furthermore, these antibodies could be removed by adsorption with purified MBP. In the present study, each patient serum sample was tested in parallel for reactivity with MBP-OspC and with MBP alone by both ELISA and Western blot. Serum samples from patients who had reactivity to MBP were retested before and after adsorption with MBP. In only 1 of the 295 samples was the reactivity to the MBP-OspC fusion protein due solely to anti-MBP antibodies. We concluded that MBP-OspC could be used in serologic assays without further modification, accompanied by the use of appropriate controls.

Antibody response to OspC during the course of Lyme disease. To investigate the natural history of the antibody response to OspC in Lyme disease, we used ELISA to test serial serum samples from 15 patients who were monitored from disease onset through 4 to 12 years of illness. As expected from previous observations (10), 14 of the 15 patients had weak to strong IgM responses to OspC in the first weeks of illness when erythema migrans or meningitis was present, and 6 of the 14 patients also had weak to moderate IgG responses (Fig. 3). Months later, following periods of minimal or no reactivity with OspC, 9 of the 15 patients developed weak to strong IgG responses to this protein during episodes of arthritis; the other 6 patients did not have responses to OspC during periods of joint involvement (Fig. 3A). The four patients with chronic arthritis, defined as 1 year or more of continuous joint inflammation, had the highest levels of IgG reactivity with OspC (Fig. 3C and D). Years later, in the two patients who developed chronic encephalomyelitis or encephalopathy after episodes of arthritis, the responses to OspC were again weak (Fig. 3B and C).

Antibody responses to OspC and manifestation of disease. To determine if the results from the patients monitored longitudinally were reproducible in a larger population, we analyzed the IgM and IgG antibody responses to MBP-OspC by ELISA in single serum samples from 189 patients with early or late manifestations of Lyme disease. As shown in Fig. 4, the IgM response to OspC was often high early in infection in patients with erythema migrans or meningitis. The IgG response to this protein was usually highest in those with arthritis from 3 months to 4 years after disease onset. This response was generally low or absent in those with chronic neuroborreliosis,

FIG. 3. Humoral immune response to OspC and the clinical course of Lyme disease. IgM and IgG antibody responses to OspC in serial sera of four representative patients, monitored for 4 to 12 years throughout the course of their illness, were determined by indirect ELISA. The clinical course is shown at the bottom of each panel. Patient A had minimal reactivity with OspC early in the illness and no reactivity during the later periods of mild arthritis. Patient B had a marked IgM response to OspC and a minimal IgG response early in the illness; a moderate IgG response was again apparent during one brief attack of arthritis 2 years later. Ten years after disease onset, when encephalomyelitis developed, the IgG response to OspC was again low. Patient C had a marked IgM response early in the illness and a moderate to high IgG response to OspC at the time of chronic arthritis. When encephalopathy developed 7 years after disease onset, the IgG response to OspC was again low. Finally, patient D had a moderate IgM response to OspC followed by an IgG response that coincided with the period of chronic arthritis.


A
IgM
IgG
Severity of Arthritis
Disease Onset
B
Encephalomyelitis
C
Encephalopathy
D
Absorbance Units
Years of Disease

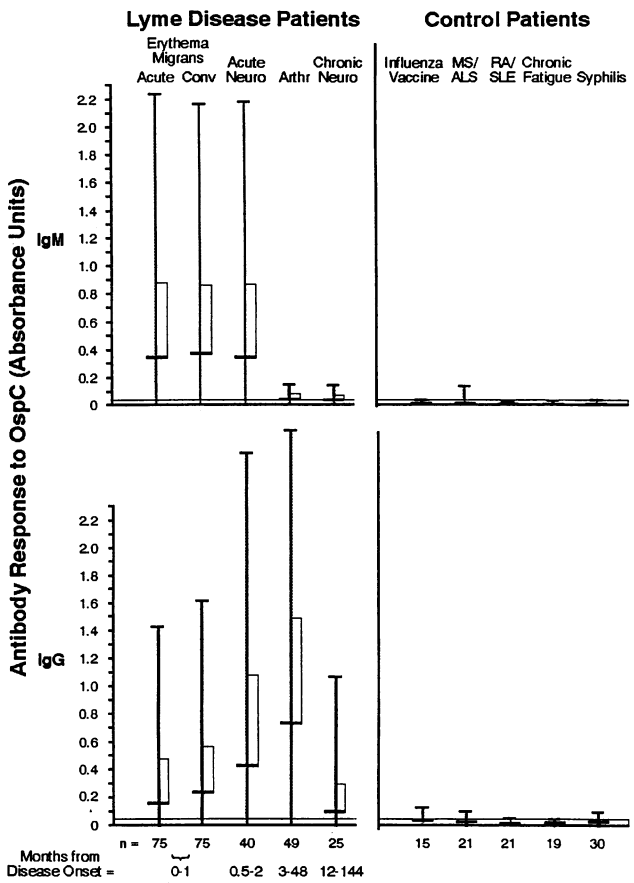


FIG. 4. Humoral immune response to OspC in patients with various manifestations of Lyme disease. IgM and IgG antibody responses to OspC in patients with early or late manifestations of Lyme disease and in control patients were determined by ELISA. The mean (horizontal bars) and range (vertical bars) of reactivity are shown; the thin brackets represent one standard deviation above the mean. The cutoff value for normal ranges was 0.03 for IgM and 0.05 for IgG, based on reactivity in 28 normal control subjects. Abbreviations: Neuro, neuroborreliosis; Arthr, arthritis; MS, multiple sclerosis; ALS, amyotrophic lateral sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; Conv, convalescent.

TABLE 1. Frequency of IgM or IgG responses to MBP-OspC by ELISA in patients with Lyme disease or other conditions

Disease or condition (no. of patients)	No. (%) of patients positive for[a]: IgM	IgG	IgM or IgG
Lyme disease			
Erythema migrans (75)			
Acute phase			
Localized (16)	4 (25)[b]	5 (31)	6 (38)
Disseminated (59)	36 (61)	20 (34)	36 (61)
Total	40 (53)	25 (33)	42 (56)
Convalescent phase			
Localized (16)	8 (50)[b]	7 (44)	10 (62)
Disseminated (59)	47 (80)	30 (51)	48 (81)
Total	55 (73)	37 (49)	58 (77)
Meningitis or facial palsy (40)	29 (72)	26 (65)	35 (88)
Arthritis (49)	22 (45)	41 (84)[c]	43 (88)
Chronic neuroborreliosis (25)	5 (20)	9 (36)	12 (48)
Controls			
Influenza vaccination (15)	0	4 (27)	4 (27)
Multiple sclerosis (12)	1 (8)	3 (25)	4 (33)
Amyotrophic lateral sclerosis (9)	0	1 (11)	1 (11)
Rheumatoid arthritis (12)	0	0	0
Systemic lupus erythematosus (9)	0	0	0
Chronic fatigue syndrome (19)	0	0	0
Syphilis (30)	1 (3)	7 (23)	7 (23)

[a] The cutoff value for positivity was based on the responses in 28 normal control subjects.
[b] For IgM, localized versus disseminated: acute, $P = 0.01$; convalescent, $P = 0.02$.
[c] For IgG, arthritis versus chronic neuroborreliosis, $P = 0.0006$.

which occurred from 1 to 12 years after disease onset. Thus, the overall pattern of OspC reactivity in this larger population was similar to that in the patients monitored serially.

To evaluate the usefulness of OspC as a diagnostic test, we next analyzed these data according to the percentage of patients with positive results at each stage of the illness (Table 1). The cutoff values for determining positive results were based on the responses in 28 normal control subjects. Of the 75 patients with erythema migrans, who were seen a mean of 7 days after the onset of symptoms, 53% had an IgM response to OspC in acute-phase sera and 73% had IgM reactivity during convalescence, 2 to 4 weeks after antibiotic treatment. Patients with early disseminated disease had a significantly higher frequency of IgM reactivity with OspC than did those with infection localized to the skin in both acute-phase (61 versus 25%; $P = 0.01$) and convalescent-phase (80 versus 50%; $P = 0.02$) sera. Considerably fewer patients with erythema migrans had IgG responses to OspC (33% in acute-phase sera and 49% during convalescence), and most also had IgM responses to this protein. Therefore, only a few more patients had positive test results if both IgM and IgG reactivity were determined at this time in the illness.

From 2 weeks to 2 months after disease onset, 72% of the 40 patients with Lyme meningitis or facial palsy had IgM responses to OspC, 65% had IgG responses, and 88% had responses to one or the other (Table 1). Of the 49 patients with Lyme arthritis who were seen months to years after disease onset, 84% had elevated IgG responses to OspC. In contrast to the arthritis patients, only 36% of the 25 patients with chronic neuroborreliosis had IgG responses to this protein ($P = 0.0006$). Low-level IgM responses were also apparent in 45% of the patients with arthritis and 20% of those with chronic neuroborreliosis. Thus, late in the illness, IgG reactivity with OspC could be demonstrated readily in patients with arthritis.

To determine the specificity of OspC determinations, we tested serum samples from 106 control patients with other conditions that may be confused with Lyme disease. Of the 106 control patients, only 1 with multiple sclerosis and 1 with syphilis had weak IgM reactivity with OspC by ELISA (Table 1). However, with this method, as many as one-quarter of the patients in certain control groups had low-level IgG reactivity with this protein. Thus, the specificity of the ELISA was good for IgM determinations but not for IgG determinations.

Sensitivity and specificity of the antibody response to MBP-OspC in early infection. Because the serodiagnosis of early Lyme disease has been a particular problem (10, 34), we calculated the sensitivity and specificity of the ELISA using MBP-OspC in patients with early infection. In the 75 patients with erythema migrans, the sensitivity of the IgM ELISA was 53% with acute-phase sera and 73% with convalescent-phase sera; the specificity was 98%, based on the responses in the 106 control patients (Table 2). When the IgG response was included in the analysis, the overall sensitivity increased (56% in acute-phase sera and 77% in convalescent-phase sera) but the

TABLE 2. Sensitivity and specificity of antibody response to MBP-OspC in early Lyme disease by ELISA and Western blotting

Test and stage of disease	% Positive IgM		% Positive IgG		% Positive IgM or IgG	
	Sens[a]	Spec[a]	Sens	Spec	Sens	Spec
ELISA						
Erythema migrans						
Acute	53	98	33	86	56	85
Convalescent	73	98	49	86	77	85
Western blot						
Erythema migrans						
Acute	59	98	47	94	65	92
Convalescent	73	98	57	94	80	92

[a] Sens, sensitivity (based on 75 patients with erythema migrans); Spec, specificity (based on 106 control patients). Both are described in Table 1.

specificity dropped substantially (85%). To determine if the sensitivity and specificity could be improved by Western blotting, we also tested case and control sera for reactivity with MBP-OspC by this method. For the IgM response, Western blotting gave similar results to those obtained by ELISA. However, for the IgG response, Western blotting resulted in better sensitivity and specificity than the ELISA did (Table 2). When the IgM and IgG results were considered together, the sensitivity of the Western blot test was 80% and the specificity was 92%.

DISCUSSION

Initial studies of the antibody response in North American Lyme disease failed to detect a response to a 22- or 23-kDa protein (3, 7). Recent studies have shown that the circular plasmid encoding the OspC lipoprotein of *B. burgdorferi* was present in all isolates tested but that not all isolates expressed the protein (24, 42). In the present study, we found that the OspC protein was expressed in our common laboratory strain of *B. burgdorferi* throughout more than 100 passages and was present in five low-passage human isolates recovered early in the infection. The variation in intensity of the OspC band on Western blots may be a result of variable expression of the protein by the spirochete, variable recovery of the protein during antigen preparation, or differences in the L22 1F8 epitope among strains. Although the molecular mass of OspC in strain G39/40 remained the same over the course of long-term passaging, the apparent molecular mass varied among the different isolates. Several groups have now documented variation among isolates in the *ospC* genes and gene products (24, 33, 42).

In a previous report, identical *ospC* sequences were found in *B. burgdorferi* B31 and PKa2, which were isolated in New York and Germany, respectively (42). In contrast, we found only 84 to 85% nucleotide identity and 76 to 79% amino acid identity among three *B. burgdorferi* strains, B31, 297, and 2591, all of which were isolated in New York. Thus, although *B. burgdorferi ospA* genes have had >99% sequence identity (11, 15), the *ospC* genes of the three *B. burgdorferi* strains discussed here were no more related to each other than to those of *B. garinii* or *B. afzelii* strains. At the protein level, most of the diversity among strains in OspC sequences occurred in the central portion of the protein. Such differences may have bearing on the efficacy of an OspC vaccine (30) or on the sensitivity and specificity of serologic tests.

Recent studies have reported that both European and North American patients with Lyme borreliosis often have prominent early responses to OspC (10, 29, 43). The present study, which examined serial sera from patients observed thoughout the course of the illness, gives a more complete picture of the antibody response to this protein in North American Lyme disease. As in previous studies, we found that most patients had weak to strong IgM responses and sometimes weak to moderate IgG responses to OspC early in the illness. However, it has not been previously noted that after months of minimal or no reactivity, some patients may develop weak to strong IgG responses to OspC that may parallel the course of the arthritis. The fact that the response to this protein was weak or absent in patients with chronic neuroborreliosis suggests that this reactivity is not simply a function of the length of infection. However, in these patients, might antibody to OspC be present in CSF rather than serum? In a previous study (19), 44% of patients with chronic neuroborreliosis had evidence of a small intrathecal antibody response to whole spirochetal lysates. This evidence is based on a comparison of the proportion of total to specific antibody in serum and CSF. However, even when there is evidence of intrathecal antibody production, the actual level of specific antibody in CSF is still about 1,000-fold lower than in serum. Thus, it is unlikely that significant amounts of antibody to OspC would be found in the CSF of these patients.

Since many of the serial sera tested in the current study had been previously tested for reactivity with OspA, it is now apparent that this pattern of reactivity is similar to the immune response to OspA (17). However, all 15 patients monitored serially had a late IgG response to OspA during maximal periods of arthritis, whereas only 9 patients (60%) had such a response to OspC. In an analysis of risk factors associated with treatment-resistant Lyme arthritis, the response to OspA was a significant risk factor for this outcome but the response to OspC was not (39). Thus, the association between OspA and arthritis is stronger than that between OspC and arthritis.

Because patients in Europe usually have an early antibody response to OspC but no later response to OspA (41), it has been hypothesized that OspC expression by European strains of *B. burgdorferi* is high and OspA expression is low (42). In America, where patients were thought to often have an early response to OspC and a late response to OspA, it has been hypothesized that OspA or OspC is expressed differentially (42). In one study of *B. burgdorferi* infection in mice, the converse was observed: the immune response shifted from a major OspA-specific response to reactivity with a 24-kDa protein (32). The current study suggests that the human antibody responses to OspA and OspC often occur together, including an early IgM response and a strong late IgG response during periods of arthritis. A recent survey of 28 representative isolates of all three species of Lyme disease spirochetes indicated that outer surface proteins A, B, and C were frequently expressed together in cultured spirochetes (6). However, the spirochetal and host factors that regulate the expression of and immunological response to these Osp proteins are not yet known.

An important goal of the present study was to assess the use of MBP-OspC in the serodiagnosis of early infection. In a previous study, a subset of the same case and control serum samples as those used in the present study were tested with spirochetal lysates as the antigen in ELISA and Western blots (10). In the ELISA, 40% of patients with erythema migrans had positive IgM test results in acute-phase sera and 72% had positive results by convalescence 2 to 4 weeks later; the specificity of the test was 94%. In Western blots, the sensitivity of the IgM blots remained the same but the specificity improved to 99%. In the present study, the sensitivity and

specificity of IgM ELISAs and Western blots were comparable or slightly improved when MBP-OspC, rather than spirochetal lysates, was used. When the IgG response to OspC was included, the sensitivity was still better but the specificity was lower, particularly in the ELISA. In this retrospective analysis, a high percentage of the patients (84%) had clinical evidence of disseminated infection. In those with infection localized to the skin or in those who are seen within the first days of symptoms, the frequency of seropositivity would surely be less than reported here. In addition, this analysis shows that the response to OspC is not completely specific for infection with *B. burgdorferi*. Recently, a genetic and antigenic homolog to OspC was found in five additional *Borrelia* species (25). Perhaps other bacteria also contain epitopes that are cross-reactive with this protein. Finally, differences in *ospC* genes among strains of *B. burgdorferi* may affect the sensitivity of serologic tests that use this antigen.

In conclusion, in early Lyme disease OspC often elicits a prominent IgM response that can be detected easily in tests involving a recombinant OspC protein. Although it is not yet clear how this antigen will compare with other recombinant antigens in the serodiagnosis of early infection, the sensitivities and specificities of the IgM ELISA and Western blot were comparable or slightly improved by using MBP-OspC rather than spirochetal lysates. Thus, OspC would seem to be a good candidate antigen, probably in combination with other early antigens, for the serodiagnosis of early infection.

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