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A 55-Kilodalton Antigen Encoded by a Gene on a *Borrelia burgdorferi* 49-Kilobase Plasmid Is Recognized by Antibodies in Sera from Patients with Lyme Disease

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We have identified a 55-kDa antigen encoded by a gene on a 49-kb plasmid of *Borrelia burgdorferi*. The screening of a *B. burgdorferi* DNA expression library (N40 strain) with rabbit anti-*B. burgdorferi* serum and then with serum from a patient with Lyme disease arthritis revealed a clone that synthesized an antigen that was reactive with both sera. DNA sequence analysis identified an operon with two genes, *s1* and *s2* (1,254 and 780 nucleotides), that expressed antigens with the predicted molecular masses of 55 and 29 kDa, respectively. Pulsed-field gel electrophoresis showed that the *s1-s2* operon was located on the 49-kb plasmid. Recombinant S1 was synthesized as a glutathione *S*-transferase fusion protein in *Escherichia coli*. Antibodies to recombinant S1 bound to a 55-kDa protein in lysates of *B. burgdorferi*, indicating that cultured spirochetes synthesized S1. Thirty-one of 100 Lyme disease patients had immunoglobulin G (IgG) and/or IgM antibodies to S1. IgG antibodies to S1 were detected by enzyme-linked immunosorbent assay and immunoblots in the sera of 21 (21%) of 100 patients with Lyme disease; 11 (27.5%) of the S1-positive samples were from patients (40) with early-stage Lyme disease, and 10 (16.7%) were from patients (60) with late-stage Lyme disease. Fifteen (38.5%) of 40 serum samples from patients with early-stage Lyme disease had IgM antibodies to S1. These data suggest that the S1 antigen encoded by a gene on the 49-kb plasmid is recognized serologically by a subset of patients with early- or late-stage Lyme disease.

Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is characterized by an initial cutaneous infection which can disseminate to involve multiple organ systems, including the heart, nervous system, and joints (34). *B. burgdorferi* is transmitted to humans by the bite of *Ixodes* ticks (34). The spirochete initially resides in the skin and must gain access to the bloodstream to cause infection resulting in carditis, arthritis, or neurologic symptoms.

B. burgdorferi antigens may play important roles in immunity and the pathogenesis of infection. The genome of *B. burgdorferi* consists of a linear chromosome (10) and a series of linear and circular plasmids (18). In *B. burgdorferi*, the genes encoding several known surface-exposed lipoproteins have been cloned. The genes for the major outer surface proteins (Osps) A and B are located on a 49-kb linear plasmid (2, 3). The *ospC* gene (17) is found on a 27-kb circular plasmid (28) and demonstrated to be actively transcribed in selected *B. burgdorferi* strains (22). The *ospD* gene is located on a 38-kb linear plasmid and preferentially expressed by low-passage, virulent *B. burgdorferi* organisms (24). An operon encoding the *ospE* and *ospF* genes is on a 42-kb plasmid (21), a 27-kDa antigen (P27) is encoded by a gene on a 55-kb linear plasmid (27), and the gene for the IpLA7 (P22) is encoded on the chromosome (20, 35).

OspA, OspB, OspC, and OspF play roles in protective immunity to *B. burgdorferi* infection. Active immunization with OspA or the passive transfer of OspA antibodies protected mice from infection with *B. burgdorferi* (11, 30). Mice immunized with OspB (12) and gerbils immunized with OspC (26) can also be protected from infection. Furthermore, we recently showed that the spirochetes were partially destroyed within ticks that engorged on OspE- or OspF-immunized mice (23).

B. burgdorferi antigens may also have some utility in diagnostic assays to identify patients with Lyme disease. Antibodies to OspC appear early after infection (8, 25). In contrast, some patients with late-stage disease developed antibodies to OspA, OspB (14), P22 (20), and OspF (23). In this study, we have identified a gene encoding a 55-kDa *B. burgdorferi* antigen (S1) that is located within a bicistronic operon on a 49-kb plasmid that encodes *ospA* and *ospB* and characterized the antibody response to S1 in sera from patients with Lyme disease.

MATERIALS AND METHODS

Cloning of the operon encoding *s1* and *s2*. A lambda ZAP II *B. burgdorferi* N40 expression library was constructed previously (21). The library was screened initially with rabbit anti-*B. burgdorferi* serum and then with serum from a patient with Lyme disease characterized by arthritis. The anti-*B. burgdorferi* serum was prepared by the intravenous injection of a rabbit with an inoculum of 10^8 live *B. burgdorferi* N40 organisms, and screening was performed with the picoBlue Immunoscreening Kit (Stratagene, La Jolla, Calif.) (21). Phages were plated on *Escherichia coli*, protein synthesis was induced with 10 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and the recombinant antigens were transferred to nitrocellulose membranes and incubated with a 1:200 dilution of antiserum. After washing, the filters were incubated with a 1:5,000 dilution of alkaline phosphatase-conjugated goat anti-rabbit or anti-human immunoglobulin G (IgG) antibodies (Organon Teknika Corporation, West Chester, Pa.), and bound antibodies were detected by color development with nitroblue tetrazolium (Stratagene) and 5-bromo-4-chloro-3-indolyl phosphate (Stratagene). Excision of the pBluescript plasmid from reactive clones was achieved with the R408 helper phage (Stratagene) (21). To sequence the insert of *B. burgdorferi* DNA, a set of deletions in the target DNA of the clone was generated with the Erase-A-Base system (Promega, Madison, Wis.). Both strands of the generated subclones were

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sequenced by the dideoxynucleotide chain-termination method (29) with the Circum Vent thermal cycle PCR sequencing kit (New England Bio-Labs, Beverly, Mass.) and analyzed with the MacVector program (Kodak, Rochester, N.Y.).

Synthesis and purification of S1 and S2. The *s1* gene lacking the sequence encoding for the hydrophobic, N-terminal leader region (amino acids 1 to 18) was amplified by PCR with oligonucleotide primers based on its DNA sequence. Elimination of the signal sequence increased the likelihood that the S1 protein would be soluble when synthesized, as described previously for the purification of OspA (9). The *s1* primers corresponded to nucleotides 55 to 75 and 1234 to 1254 of the *s1* gene. The entire *s2* gene was also PCR amplified, with the *s2* primers corresponding to nucleotides 1 to 21 and 760 to 780 of the *s2* gene. The template, DNA from an original reactive clone, was denatured at 94°C for 1 min, annealed at 67°C for 1 min, and extended at 72°C for 1 min. This process was repeated for 30 cycles. The amplified *s1* and *s2* genes were cloned in frame with the glutathione *S*-transferase (GT) gene into pMX, a pGEX-2T vector (Pharmacia, Piscataway, N.J.) with a modified polylinker (32). The PCR-amplified DNA sequences of *s1* and *s2* were confirmed by sequencing both strands of the DNA.

DH5α cells containing the recombinant plasmids were grown to an optical density at 600 nm of 0.5 (about 2 h), and the recombinant fusion proteins were induced by the addition of IPTG to a final concentration of 1 mM (2 h). The bacterial cells were centrifuged at 4,000 rpm (Beckman J-6B) for 20 min, and the pellet was washed with phosphate-buffered saline (PBS) and dissolved in 1/10 volume of PBS with 1% Triton X-100. The mixture was sonicated and centrifuged at 10,000 rpm. The Coomassie blue-staining gels showed that the GT-S1 recombinant fusion protein was soluble and in the supernatant. The supernatant containing the GT-S1 protein was loaded onto a glutathione-Sepharose 4B column (Pharmacia), and the GT-S1 protein was eluted with 10 mM GT in 50 mM Tris-HCl buffer (pH 8.0) (11).

Pulsed-field gel electrophoresis. Pulsed-field gel electrophoresis was performed with total *B. burgdorferi* N40 DNA as described previously, with minor modifications (10). DNA plugs containing approximately 10^8 *B. burgdorferi* N40 organisms were loaded onto an 0.8% agarose gel which was run in Tris-borate-EDTA buffer (0.025 M Tris, 0.5 mM EDTA, 0.025 M boric acid) by use of the CHEF-DRII system (Bio-Rad Laboratories, Richmond, Calif.). The gel was run at 14°C for 18 h at 198 V, with ramped pulse times from 1 to 30 s. Southern blotting with *s1* or *s2* DNA as the probe was carried out as described previously (33).

Production of anti-GT-S1 sera and immunoblots. C3H/HeJ mice (Jackson Laboratory, Bar Harbor, Maine) were immunized subcutaneously with 10 μg of purified GT-S1 in complete Freund's adjuvant and boosted at 14 and 28 days with the same amount in incomplete Freund's adjuvant. Mice were bled 2 weeks after the last boost to collect the antisera.

GT-S1 or lysates of *B. burgdorferi* were resolved in a sodium dodecyl sulfate (SDS)–12% polyacrylamide gel by electrophoresis and transferred to nitrocellulose membranes which were cut into strips. The S1 strips and *B. burgdorferi* lysate strips were probed with murine anti-GT-S1 sera. The secondary antibody was alkaline phosphatase-conjugated goat anti-mouse IgG (Stratagene).

ELISA. Two hundred microliters of a 1-μg/ml concentration of GT-S1 in carbonate coating buffer (pH 9.6) was plated in 96-well plates (ICN, Costa Mesa, Calif.) (7) for 90 min at room temperature and then washed with PBS–0.1% Tween 20 (PBST) three times. Human sera were diluted with PBST (1:100) and added to plates at 37°C for 45 min. The plates were washed three times with PBST. Goat anti-human IgG or IgM linked with alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.; 1:10,000) was added to the plates at 37°C for another 45 min. The plates were washed with PBST three times, and 1 mg of *p*-nitrophenyl phosphate per ml was added to the plates. The plates were incubated at 37°C for 1 h and read on a Titertek Multiskan (ICN). The range of enzyme-linked immunosorbent assay (ELISA) units was from 100 to 6,400. A measurement of 400 U or higher was considered positive.

Human serum. Sera from patients with early- or late-stage Lyme disease were collected as described previously (14). Since the time of occurrence of the tick bite was unknown, patients who had erythema migrans and skin lesions for less than 1 week were classified as early-stage disease patients, and those who had previous erythema migrans and arthritis for at least 6 months were classified as late-stage disease patients (14). Late-stage Lyme disease serum samples were obtained 1 to 2 years after the initial diagnosis. All sera that reacted with whole-cell lysates of *B. burgdorferi* in an ELISA were selected.

Nucleotide sequence accession numbers. *s1* and *s2* sequence data have been submitted to the GenBank nucleotide sequence databases. The accession numbers for *s1* and *s2* are L34016 and L34017, respectively.

RESULTS

Cloning the operon which encodes *s1* and *s2*. The *B. burgdorferi* N40 genomic DNA expression library was screened with rabbit anti-*B. burgdorferi* N40 serum and then with serum from a patient with late-stage Lyme disease. A clone expressing a *B. burgdorferi* N40 antigen that reacted with both sera was isolated for further study. The DNA sequence obtained by sequencing both strands of the clone revealed a putative bicistronic operon which encoded two genes, designated *s1* and *s2*. Figure 1 shows the nucleotide sequences of *s1* and *s2* and their 5′ and 3′ flanking regions as well as the predicted amino acid sequences of the encoded proteins. The first open reading frame (*s1*) contains 1,251 nucleotides and is separated from the second open reading frame (*s2*) by 181 nucleotides. *s2* is 780 nucleotides in length, and its stop codon, TAA, is followed by a putative hairpin structure, the potential terminator of transcription. A consensus ribosome binding site with the sequence AGAGG is 16 bp upstream of the start codon of the *s1* gene, and a −10 region, TATTAA, and −35 region, TTGTTT, are 123 and 149 bp upstream, respectively. The −10 and −35 putative promoters are highly homologous to the similar regions in other *B. burgdorferi* genes (21). Indeed, *s1* was expressed in *E. coli* from the *B. burgdorferi* promoter because the antigen was not fused with β-galactosidase, similar to our initial identification of OspE and OspF (21). There is another potential ribosome binding site with the sequence AGA 16 bp upstream of the start codon of the *s2* gene. A Northern (RNA) blot of *B. burgdorferi* RNA incubated with *s1* and *s2* probes revealed a band of approximately 2.2 kb, indicating that *s1* and *s2* are expressed from a bicistronic operon (data not shown). The G+C contents of the *s1* and *s2* genes are 30.7 and 31.5%, respectively, similar to the G+C content of whole *B. burgdorferi* DNA (30%) (19, 31).

The S1 protein predicted from the sequence is 417 amino acids, and its calculated molecular mass is 54.8 kDa. S2 consists of 260 amino acids and has a predicted molecular mass of 29.4 kDa. Both proteins have a relatively high content of lysine, 10.94% in S1 and 10.38% in S2, similar to the lysine contents of OspA and OspB (15%) (3), OspE (14.5%), and OspF (17.7%) (21). S1 also has a large amount of phenylalanine (10.55%). S2 has a large amount of serine (11.15%), both of which are similar to the levels in OspA and OspB (10.5%) (3). Both proteins are basic; their estimated isoelectric points are 11.53 (S1) and 9.13 (S2). A hydrophilicity profile of S1 is similar to those of OspA, -B, -C, -D, -E, and -F, suggesting that S1 may be a surface protein (Fig. 2). The amino-terminal sequence of S1 revealed the following three characteristics of signal peptides: (i) a basic amino terminus, namely, Met, Asn, and Lys (amino acids 1 to 3); (ii) a hydrophobic central core (amino acids 4 to 17); and (iii) a Leu-X-Y-Z-Cys (positions 14 to 18) consensus sequence at the carboxyl terminus of this hydrophobic domain that may be recognized by signal peptidase II (3). S1 and S2 have no significant homology with one another or other proteins at the amino acid level when compared with other proteins in GenBank.

***s1* and *s2* genes are located on a 49-kb plasmid.** To localize the *s1* and *s2* genes, we performed pulsed-field gel electrophoresis with total *B. burgdorferi* N40 DNA (Fig. 3). As expected, the flagellin gene was located on the chromosome (Fig. 3, lane 1) (4), and *ospA* was located on a 49-kb plasmid (Fig. 3, lane 2) (1). Interestingly, the *s1* (Fig. 3, lane 3) and *s2* (data not shown) DNA probes hybridized to a band at the identical position as *ospA*, suggesting that they may be on the same 49-kb linear plasmid.

Synthesis, purification, and immunogenicity of S1. *E. coli* DH5α cells containing the vectors with the *s1* or *s2* gene (pMX-S1 and pMX-S2) were induced with IPTG to synthesize the recombinant fusion proteins. Coomassie blue-staining gels indicated that the GT-S1 protein was soluble and present in the supernatant; therefore, it could be purified by use of a glutathione column (Fig. 4, lane 1). However, the GT-S2 recombinant protein was not soluble and was found in the cell

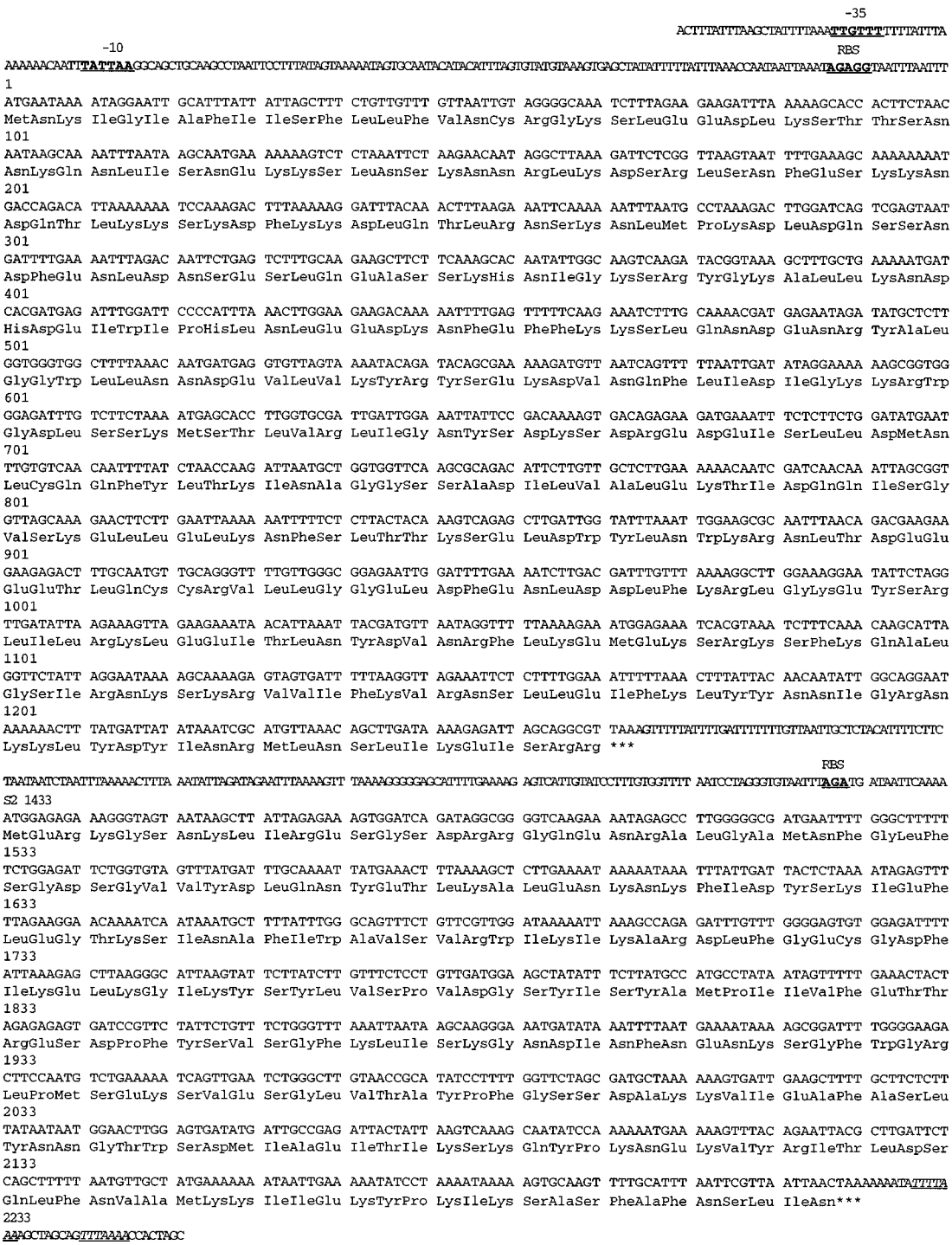

FIG. 1. *s1* and *s2* DNA and amino acid sequences. The −35 and −10 regions and putative ribosome binding sites (RBS) of *s1* and *s2* are indicated by underlining and boldface letters. Stop codons after *s1* and *s2* are indicated by asterisks. A possible hairpin structure at the end of the *s2* sequence is indicated by underlining and italic letters.

extract. Therefore, GT-S2 could not be purified by use of a glutathione column.

We then immunized mice with GT-S1 to obtain anti-GT-S1 serum. Mouse anti-GT-S1 serum recognized recombinant GT-S1 (Fig. 4, lane 6). The lower bands are most likely the degradation products of the GT-S1 protein and are analogous to similar bands that we had observed with recombinant GT-OspA or GT-OspB (13, 15). To determine whether S1 was synthesized in *B. burgdorferi* N40, we probed *B. burgdorferi* whole-cell extracts with mouse anti-GT-S1 serum. Figure 5 shows that S1 was synthesized by cultured *B. burgdorferi* and detectable when probed with mouse anti-GT-S1 serum at a

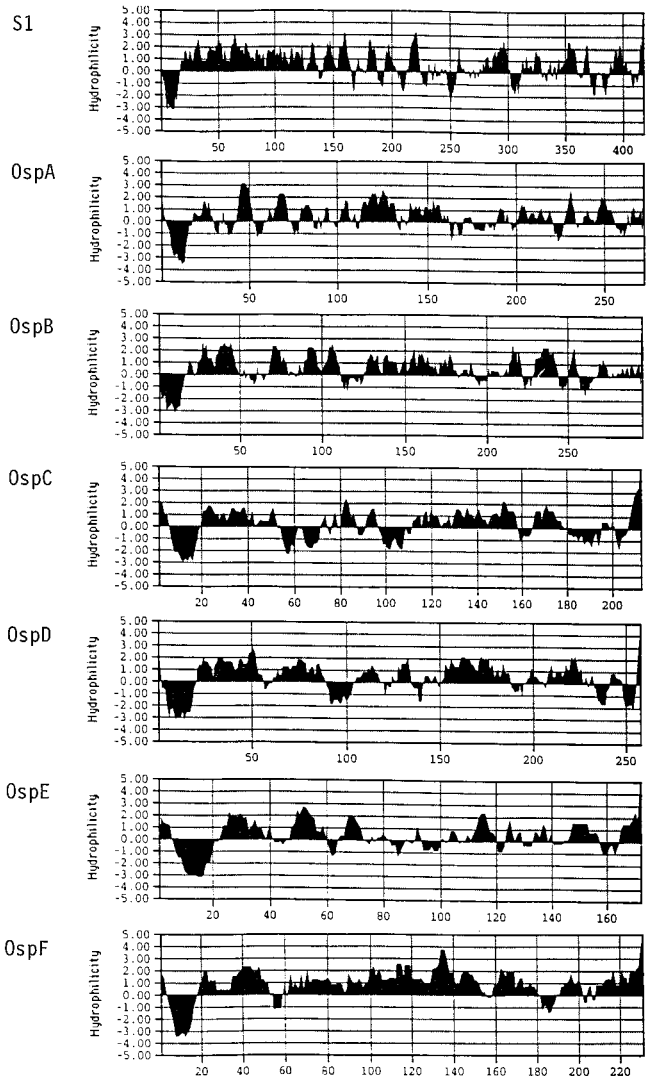

FIG. 2. Hydrophilicity (Kyte-Doolittle) profile of S1. The S1 profile is compared with profiles of OspA, -B, -C, -D, -E, and -F.

1:10,000 dilution. In addition, the mouse anti-GT-S1 bound a 80-kDa antigen as well. Both the mouse anti-GT serum and anti-GT-S1 serum bound a 46-kDa antigen, indicating background reactivity of the Enhanced Chemiluminescence detection system. S1 was also expressed by *B. burgdorferi* N40 passaged 5, 10, 30, and 50 times in BSK medium in our laboratory (Table 1).

We also determined whether S1 was synthesized by *B. burgdorferi* strains from different geographic regions and within the three genospecies, *B. burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii* (Table 1). *B. burgdorferi* sensu stricto strains 25015 (New York) (13), California 1 (16), and Northeast 2 (16) all synthesized the 55-kDa S1 protein and the 80-kDa antigen. *B. burgdorferi* California 3 (16) synthesized only the 80-kDa antigen. S1 and the 80-kDa band were not detected in the California 2 (16) or the Northeast 1 (16) strain. Among several *B. afzelii* isolates, strain Sweden 1 (16) and VS461 (Switzerland) (36) synthesized both S1 and the 80-kDa band, pKo (Germany) (36) expressed only 55-kDa S1, and Germany 1 (16) did not synthesize either band. *B. garinii* pBi (Germany) (36) did not synthesize either band.

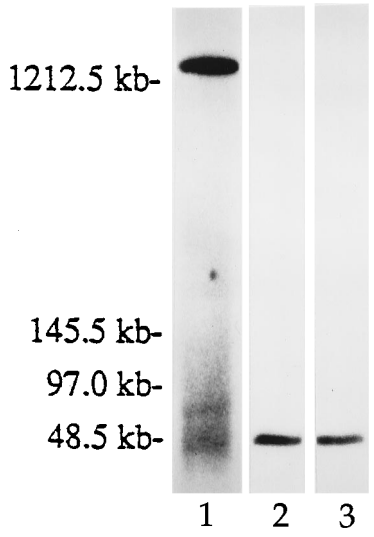

FIG. 3. Southern blot of *B. burgdorferi* N40 chromosomal and plasmid DNA separated by pulsed-field gel electrophoresis and probed with flagellin (lane 1), *ospA* (lane 2), and *s1* (lane 3). The molecular mass standards are indicated on the left.

To determine whether S1 was processed by *B. burgdorferi* cells as a lipoprotein because of its Leu-X-Y-Z-Cys consensus sequence, we labeled spirochetes with [^{3}H]palmitate as described in published protocols (21) and performed an immunoprecipitation with mouse anti-S1 serum. No band consistent with a [^{3}H]palmitate-labeled S1 antigen could be detected, whereas control studies using OspA or OspE antibodies yielded the expected bands consistent with immunoprecipitated, [^{3}H]palmitate-labeled OspA or OspE (data not shown). Identical results were obtained in three separate studies, including experiments in which the autoradiographs were exposed for 2 weeks, prior to development, to detect faint bands. S1 antibodies were capable of immunoprecipitating unlabeled

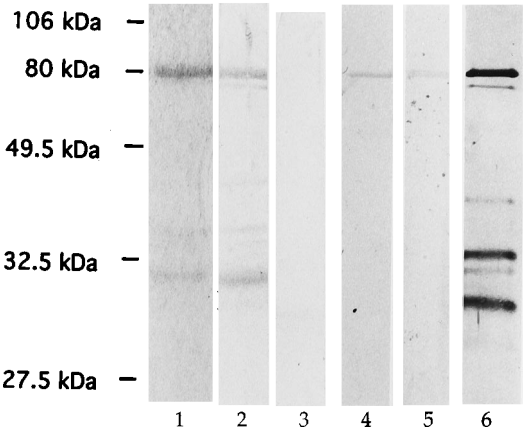

FIG. 4. Immunoblot analysis of the GT-S1 fusion protein. Lanes: 1, Coomassie blue-stained SDS-polyacrylamide gel showing the purified 78-kDa GT-S1; 2, GT-S1 probed with serum from a patient with early-stage Lyme disease; 3, GT (control) probed with serum from the patient with early-stage Lyme disease; 4, GT-S1 probed with serum from a patient infected with *B. hermsii*; 5, GT-S1 probed with serum from a rabbit immunized with *B. hermsii*; 6, GT-S1 probed with mouse anti-GT-S1 serum.

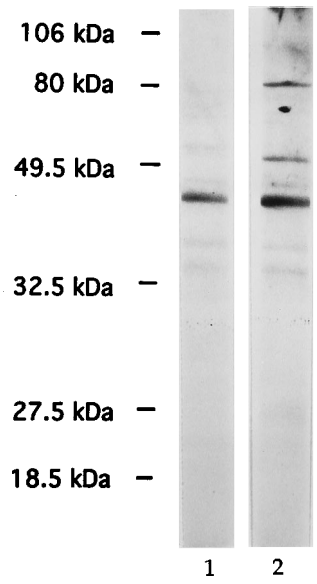

FIG. 5. *B. burgdorferi* whole-cell lysate probed with mouse anti-GT-S1 serum. Lanes: 1, mouse anti-GT serum (control); 2, mouse anti-GT-S1 serum.

S1. These data suggest that S1 may not be processed as a lipoprotein.

Evaluation of the humoral response to S1 and S2 in patients with Lyme disease. We evaluated the humoral response to S1 in humans by ELISA and immunoblots. Plates were coated with GT-S1, and GT-S1 was probed with carefully selected sera from the Yale Lyme disease reference laboratory that responded to *B. burgdorferi* whole-cell extracts. Sera from 21 of 100 patients had IgG antibodies to S1; 11 of these 21 serum samples were from patients with early-stage disease, and 10 of these serum samples were from patients with late-stage disease (Table 2). Forty of the 100 patients had early-stage disease, and 60 had late-stage disease. Of patients with early- and late-stage disease, 27.5% and 16.7%, respectively, had IgG antibodies to S1. A representative IgG immunoblot is shown in Fig. 4, lane 2, and as expected, patient serum did not react with the GT control (Fig. 4, lane 3). We also examined IgM antibodies to S1 in the sera of patients with early-stage Lyme disease since late-stage disease patients rarely have IgM antibodies to *B. burgdorferi* whole-cell lysates (Table 2). Fifteen samples (37.5%) contained IgM antibodies to S1. Indeed, 10 of these serum samples did not have IgG antibodies to S1. Therefore, overall, 31 of 100 patients had IgG and/or IgM antibodies to S1. In general, the mean antibody titers to *B. burgdorferi* lysate were higher than the mean titers to the GT-S1 fusion protein (Table 2).

TABLE 1. S1 expression by different *B. burgdorferi* strains[a]

Borrelia strain	Expression of:	
	S1	80-kDa antigen
B. burgdorferi sensu stricto		
N40 (New York)		
In vitro passage 5	+	+
In vitro passage 10	+	+
In vitro passage 30	+	+
In vitro passage 50	+	+
25015 (New York)	+	+
California 1	+	+
California 2	−	−
California 3	−	+
Northeast 1	−	−
Northeast 2	+	+
B. afzelii		
Germany 1	−	−
pKo (Germany)	+	−
Sweden 1	+	+
VS461 (Switzerland)	+	+
B. garinii pBi (Germany)	−	−

[a] Whole-cell lysates were probed with murine anti-GT-S1 serum.

We used whole-cell lysates of *E. coli* synthesizing GT-S2 (Fig. 6A, lane 2) to perform immunoblots to examine whether patients with Lyme disease had antibodies to S2. *E. coli* whole-cell lysates (Fig. 6A, lane 1) served as the control antigen. No humoral responses to the recombinant S2 were detected in 20 patients with early-stage Lyme disease and 20 patients with late-stage Lyme disease. Rabbit anti-*B. burgdorferi* serum reacted with recombinant GT-S2, indirectly showing that S2 is synthesized by *B. burgdorferi* (Fig. 6B).

We then determined whether sera from S1-positive patients had antibodies that bound to selected *B. burgdorferi* antigens, including the 31-kDa (OspA), 34-kDa (OspB), 41-kDa (flagellin), and a 22-kDa (OspC) antigens. Of 31 S1-positive patients, 2 (10%) had antibodies to OspC, 12 (39%) had antibodies to OspA, 14 (45%) had antibodies to OspB, and 27 (89%) had antibodies to flagellin when sera were blotted with *B. burgdorferi* whole-cell lysates. We then determined the clinical courses of the patients that had antibodies to S1 by contacting the referring physicians. Detailed information was available for 21 of the 31 patients. Six of the 21 patients (28.6%) had early localized disease, marked by erythema migrans; 6 (28.6%) had disseminated early disease, with multiple lesions of erythema migrans and constitutional symptoms including muscle ache, headache, and weakness; 6 (28.6%) had acute Lyme disease arthritis; 2 patients (9.5%) had Lyme carditis, marked by conduction defects and bradycardia; and 1 (4.8%) had chronic intermittent Lyme disease arthritis.

To determine the specificity of S1 for Lyme disease, we evaluated sera from patients infected with other spirochetes, e.g., syphilis and *Borrelia hermsii*, and from patients with lupus by immunoblots. Five patients with syphilis and nine patients with lupus did not have IgG antibodies to S1. However, one patient with relapsing fever caused by *B. hermsii* (Fig. 4, lane 4) and a rabbit immunized with *B. hermsii* had IgG antibodies that cross-reacted with S1 (Fig. 4, lane 5).

DISCUSSION

An operon encoding two novel *B. burgdorferi* genes, *s1* and *s2*, has been cloned. The genes share the same two −10 and −35 promoters, and they each have a putative ribosome binding site upstream of their start codons. There is a hairpin structure, the potential terminator of transcription, downstream of the stop codon of the *s2* gene, and there is no such structure downstream of the stop codon of the first gene, *s1*. The identification of a 2.2-kb band in a Northern blot of *B. burgdorferi* probed with *s1* or *s2* indicated that the genes are encoded on a bicistronic operon. In the *ospA-ospB* (31) and *ospE-ospF* operons, both antigens are lipoproteins. In the *s1-s2* operon, however, although S1 has a Leu-X-Y-Z-Cys consensus sequence from amino acids 14 to 18, suggesting that it is a putative lipoprotein, incorporation of [^{3}H]palmitate into S1 was not detected, implying that S1 may not be a lipoprotein. Furthermore, OspA and OspB have 55% homology (3), and

TABLE 2. Reactivity of human sera to whole-cell lysates of *B. burgdorferi* or purified recombinant GT-S1 in ELISA

Disease stage	No. of patients	Patients positive by ELISA							
		B. burgdorferi				GT-S1			
		IgG		IgM		IgG		IgM	
		No. (%)	Mean (range)	No. (%)	Mean (range)	No. (%)	Mean (range)	No. (%)	Mean (range)
Early	40	22 (55)	842 (400–6,400)	35 (87.5)	1,326 (400–6,400)	11 (27.5)	1,069 (400–6,400)	15 (38.5)	670 (400–3,200)
Late	60	59 (98.3)	2,851 (400–6,400)	NA[a]		10 (16.7)	500 (400–1,600)	NA	
Total	100	81 (81)		NA		21 (21)		NA	

[a] NA, not available.

OspE and OspF are similar within the first 30 amino acids. In contrast, S1 and S2 do not have significant sequence homology.

Data from Southern blots indicated that the plasmids carrying *s1-s2* and *ospA-ospB* operons comigrated at the identical position, suggesting that the *s1-s2* and *ospA-ospB* operons might be located on the same 49-kb linear plasmid. Recent studies by Sadziene and her colleagues have identified *B. burgdorferi* strains that do not express OspA and/or OspB and that lack the 49-kb plasmid, and some of these types of strains may be less infectious in mice (28). Our data suggest that lack of the 49-kb plasmid may result in the loss of several *B. burgdorferi* antigens, and the relative contribution of each antigen to spirochete infectivity remains to be explored.

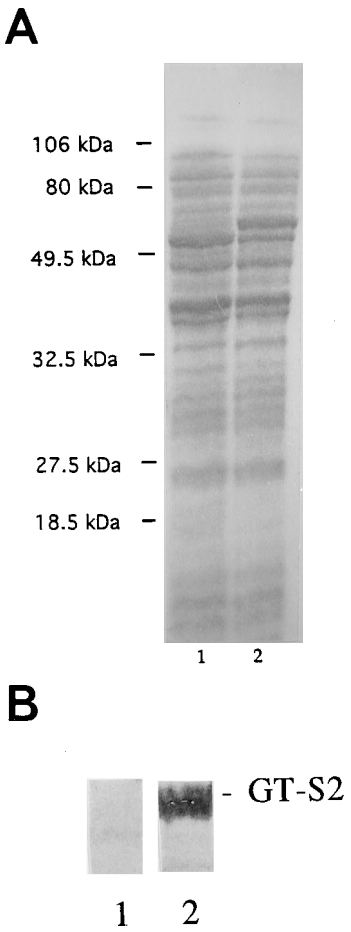

FIG. 6. (A) Coomassie blue-stained SDS-polyacrylamide gel of the recombinant GT-S2 protein synthesis in *E. coli*. Lanes: 1, *E. coli* whole-cell lysate; 2, whole-cell lysate of *E. coli* synthesizing GT-S2 (the new band halfway between the 49.5- and 80-kDa markers is the GT-S2 fusion protein). (B) Immunoblot of *E. coli* synthesizing recombinant GT-S2 probed with rabbit anti-*B. burgdorferi* serum. Lanes: 1, Enlargement of the region between 49.5 and 80 kDa of the *E. coli* whole-cell lysate; 2, enlargement of the region between 49.5 and 80 kDa of the *E. coli* lysate synthesizing GT-S2.

Immunoblots with mouse anti-GT-S1 serum indicate that *B. burgdorferi* N40 expresses a 55-kDa protein. Our results also indicate that S1 is synthesized at a very low level in cultured *B. burgdorferi* N40 because Coomassie blue staining of gels could not identify a discrete band consistent with the size of S1. In addition, S1 could not be detected on fixed N40 spirochetes with mouse anti-GT-S1 serum by indirect immunofluorescence microscopy (data not shown), further suggesting that the level of *s1* expression in spirochetes is low. We tested different strains of *B. burgdorferi*, selected from distinct genospecies, to determine whether S1 was synthesized by diverse strains. Interestingly, distinct spirochetes within *B. burgdorferi* sensu stricto and *B. afzelii* synthesized S1, indicating that expression is not limited to a single group. Moreover, the synthesis of the 80-kDa antigen that cross-reacts with S1 antibodies was detected in these two groups. Furthermore, individual organisms that did not synthesize S1 and/or the 80-kDa antigen were identified within each genospecies. These data indicate that as with other *B. burgdorferi* antigens, variability between and within the genospecies is evident. Researchers have noted that *B. burgdorferi* may undergo genotypic and phenotypic changes upon passage through culture in vitro. We have detected S1 in N40 passaged up to 50 times, indicating that S1 is not readily lost within *B. burgdorferi* N40 upon in vitro culture.

We also studied sera from patients with Lyme disease to determine whether they developed antibodies to S1. Twenty-one percent of the patients developed IgG antibodies to S1. Twenty-eight percent of the early-stage patients and 16.7% of late-stage patients had IgG antibodies. Fifteen patients with early-stage disease (38.5%) developed IgM antibodies to S1, although 10 of them did not have IgG antibodies to S1. Therefore, 31% of all the patients tested had antibodies to S1. Among 21 of the 31 S1-positive patients from whom we were able to obtain a well-characterized clinical history from the referring physicians, 28.6% had early disease, 28.6% had disseminated early disease, 28.6% had acute Lyme arthritis, 9.5% patients had Lyme carditis, and 4.8% had chronic Lyme arthritis. These data show that antibodies to S1 are not restricted to any specific disease course.

Immunoblots of *B. burgdorferi* N40 lysates probed with patient sera indicated that among S1-positive patients, 39% had antibodies to OspA, 45% had antibodies to OspB, and 97% had antibodies to flagellin. P39 could be distinguished readily from 41-kDa flagellin in our immunoblots; therefore, we did not know whether the patients had antibodies to P39. Only two (6.5%) patients had a humoral response to OspC; these were patients with early-stage Lyme disease. We detected responses

TABLE 3. Clinical illness and antibody reactivity to OspA, -B, -C, and flagellin among S1-positive patients

Clinical disease group	Patient no.	ELISA for S1		Immunoblots (IgG and/or IgM)			
		IgG	IgM	OspA	OspB	OspC	41-kDa
Erythema migrans	1	+	+	+	+	+	+
	2		+	+	+	+	+
	3		+	+	+		+
	4	+					+
	5	+	+				+
	6	+					+
Early disseminated disease	7	+			+		+
	8		+		+		+
	9		+	+			+
	10		+				+
	11	+					+
	12	+					+
Acute Lyme disease arthritis	13		+				+
	14		+	+	+		+
	15	+					+
	16	+		+	+		+
	17	+		+	+		+
	18	+		+	+		+
Lyme disease carditis	19	+					+
	20		+	+	+		+
Chronic Lyme disease arthritis	21	+		+	+		+

to OspC in two of six patients with early localized disease (Table 3), probably because the strain *B. burgdorferi* 297 used in our immunoblots does not synthesize high levels of OspC uniformly. Of 40 patients we tested, none had humoral responses to S2, suggesting that S2 is not immunogenic during infection.

Late-stage disease patients may develop antibodies to many *B. burgdorferi* proteins (5, 6, 8), including the 18-kDa protein, OspA, OspB, OspC, OspE, OspF, the 39-kDa protein, flagellin, 58-kDa, and 66-kDa heat shock proteins, and 83-kDa antigens. However, early-stage disease patients develop a more restrict response, including antibodies to OspC (8) and flagellin. No individual *B. burgdorferi* antigen is currently fully effective as a substrate in diagnostic assays. Our data suggest that S1 is recognized by some patients with Lyme disease both in the early and the late stages of infection. Moreover, like the results with OspA, OspB, and OspC, not all of the patient sera recognized S1. Therefore, a cocktail of relevant antigens may be needed for an accurate diagnostic assay.

Our data showed that sera from patients with syphilis or lupus did not cross-react with S1. However, sera from a patient infected with *B. hermsii* and a rabbit immunized with *B. hermsii* had S1 cross-reactivity. These data indicate that antibodies to S1 are specific for borrelial infection; however, the antibodies may not be able to differentiate between borrelial species. In summary, S1 is a novel 55-kDa *B. burgdorferi* antigen that is synthesized at low levels in cultured spirochetes and recognized by some patients with early- or late-stage Lyme disease.

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