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110-Kilodalton Recombinant Protein Which Is Immunoreactive with Sera from Humans, Dogs, and Horses with Lyme Borreliosis

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***Eco*RI-digested DNA from *Borrelia burgdorferi* was ligated into the dephosphorylated vector pWR590 and transformed into *Escherichia coli* DH5α. When the gene library was screened, 20 clones reacted with pooled dog sera with high titers (immunofluorescent antibody titer, ≥1,280) to this spirochete. One clone expressed a 110-kDa antigen that reacted strongly with the high-titered pooled sera from dogs with Lyme borreliosis and serum from goats immunized with *B. burgdorferi*. The 110-kDa protein was expressed with and without isopropyl-β-D-thiogalactosidase, indicating the protein is not a fusion protein with β-galactosidase. Monospecific antisera to the 110-kDa antigen recognized a 75-kDa *Borrelia* protein. Of the sera that reacted with *B. burgdorferi* by immunoblotting; 57, 100, and 83% of human, dog, and horse serum samples, respectively, reacted with the 110-kDa protein. Sera from individuals that tested negative with a *B. burgdorferi* lysate with immunoblotting showed no reaction with the 110-kDa protein. The 110-kDa antigen appears to be useful for the diagnosis of Lyme borreliosis.**

Borrelia burgdorferi is the causative agent of Lyme borreliosis. The disease in humans often begins with an early primary skin lesion called erythema migrans, which may be followed by cardiac, neurologic, or arthritic symptoms (14, 24, 32, 33, 36–39). Clinical signs in dogs and horses may include fever, anorexia, and lameness (10, 11, 22, 25, 28, 31).

To determine which antigens of *B. burgdorferi* are important in the diagnosis of and protection from Lyme borreliosis, one may purify and characterize them. Antigens can be isolated by immunoprecipitation, extraction from sodium dodecyl sulfate (SDS)-polyacrylamide gels, or molecular cloning and expression. The first two methods require large numbers of organisms, creating a logistical problem with *B. burgdorferi*, which has a generation time of 8 to 24 h at 32°C and reaches a maximum cell density of 10^7 to 10^8 cells per ml (1). Molecular cloning of protein antigens in a host such as *Escherichia coli* results in their production in large amounts without contamination by other spirochete antigens. The use of *E. coli* as a host for molecular cloning also avoids association of *B. burgdorferi* antigens with rabbit serum, since the bacterium is grown in Luria broth (LB) containing no serum. This is critical in the development of vaccines, since spirochetal antigens may adsorb rabbit serum, a component of the medium used to propagate *B. burgdorferi*, potentially resulting in anaphylactic shock when administered to animals (1, 40).

Molecular and immunological characterization of *B. burgdorferi* has led to a better understanding of the components that may be useful for the diagnosis of or protection against Lyme borreliosis. Two major surface proteins with molecular masses of 31 and 34 kDa (OspA and OspB, respectively) have been cloned, sequenced, and characterized (5, 6, 20). Those two proteins are under the control of a single promoter and are found on a linear plasmid (3, 19). Fikrig et al. reported that mice injected with purified OspA or with hyperimmunized serum to OspA were protected from joint swelling, carditis, and infection with *B. burgdorferi* (15). The 41-kDa protein, a flagellin component, has been cloned and sequenced and found to be recognized early in the immune response (2, 13, 41). Unfortunately, antibodies specific to this flagellin component cross-react with other species of *Borrelia* (4, 16). A fourth immunodominant protein of 60 kDa has also been cloned (18). Antibodies to this recombinant protein cross-react with a large variety of microorganisms, including *Pseudomonas* and *Legionella* spp.; therefore this antigen is not useful for the diagnosis of Lyme borreliosis.

The purpose of this study was to clone and characterize a *B. burgdorferi* protein that reacts with antibodies from humans and animals with Lyme borreliosis.

MATERIALS AND METHODS

Bacterial organisms. The *B. burgdorferi* MM1 isolate came from a kidney of *Peromyscus leucopus* in Minnesota (26). *B. burgdorferi* B31, *Borrelia hermsii* HS1, *Borrelia anserina*, and *Treponema denticola* were kindly provided by R. C. Johnson, University of Minnesota. Isolate B31 originated from a tick (*Ixodes dammini*) from Shelter Island, N.Y. (9). *Leptospira interrogans* serovars canicola and pomona were provided by the Veterinary Diagnostic Laboratory, University of Minnesota. *E. coli* DH5α was obtained from Bethesda Research Laboratories, Inc., Gaithersburg, Md.

Animals. Male and female CF-1 strain white mice, each weighing 20 to 25 g, were obtained from Jackson Laboratories (Bar Harbor, Maine).

DNA purification. All spirochetes were cultured in BSK-II medium to the stationary phase (approximately 10^8 cells per ml) at 32°C (1). *T. denticola* was grown at 34°C in a medium described by Smibert (35), and *Leptospira* spp. were grown at 30°C in a medium developed by Bey and Johnson (7). Cells were recovered by centrifugation, washed once in 10 ml of TES (50 mM Tris-HCl [pH 8.0], 10 mM EDTA, 50 mM NaCl), and suspended in 5 ml of TES with 50 mg of RNase A per ml. Lysozyme was added to a final concentration of 2 mg/ml. After 20 min at 37°C, 20 μl of proteinase K (25 mg/ml) and 5 ml of 10% SDS were added, and the mixture was

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incubated for 12 to 15 h at 37°C. DNA was extracted once with phenol, three times with phenol-chloroform, and then with chloroform. The aqueous phase was removed and mixed with 0.5 volume of 7.5 M ammonium acetate, and the DNA was precipitated with 0.6 volume of isopropanol. The DNA precipitate was collected by a 15-min centrifugation at 12,000 × *g*, washed thoroughly with 70% (vol/vol) ethanol, and resuspended in TE (10 mM Tris-HCl [pH 8.0], 1 mM EDTA).

Library construction and screening. Genomic libraries were constructed as described by Maniatis et al. (30). Briefly, *B. burgdorferi* MM1 DNA was partially digested with *Eco*RI, and 2- to 8-kb fragments were isolated on a 10 to 40% (wt/vol) sucrose gradient. DNA fragments were ligated with T4 DNA ligase (Bethesda Research Laboratories) to the plasmid vector pWR590 (17), which was dephosphorylated with calf intestinal alkaline phosphatase (Promega Biotech, Madison, Wis.). Recombinant plasmids were used to transform *E. coli* DH5α, and transformants were selected by growth on LB plates containing 100 μg of ampicillin per ml. Colonies were transferred by replica plating to nitrocellulose filters (HATF, 0.45-μm pore size; Millipore Corp., Bedford, Mass.). For the induction of a fusion protein, 0.5 mM isopropyl-β-D-thiogalactosidase (IPTG; Sigma Chemical Co., St. Louis, Mo.) was added to an 18th culture of *E. coli* in LB broth, and the culture was incubated for 8 h at 37°C.

The genomic library was screened immunologically as described by Young and Davis (42). Replicate membrane preparations each containing approximately 3,000 colonies were lysed by chloroform vapor for 15 min and then treated with a buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM $MgCl_2$, 5% nonfat dried milk (NFDM), 2 μg of DNase, and 40 μg of lysozyme per ml overnight at room temperature with shaking. Filters were rinsed with Tris-saline (50 mM Tris-HCl [pH 7.5], 150 mM NaCl) and 5% NFDM to remove any remnants of colonies. Primary antibody (sera from dogs originating from east central Minnesota and western Wisconsin that had an immunofluorescent antibody [IFA] titer of ≥1,280 against *B. burgdorferi*, a history of tick exposure, and lameness and responded to antimicrobial therapy) was diluted to 1:100 and preabsorbed with a boiled lysate of *E. coli* DH5α overnight at 4°C. The filters were incubated at 4°C overnight with diluted and preabsorbed primary antibody and washed six times with Tris-saline–5% NFDM–0.05% Nonidet P-40 (Sigma). Filters were incubated with a biotin-labeled goat anti-dog immunoglobulin IgG (heavy and light chains) diluted 1:250 (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) for 1 h at room temperature. The filters were washed five times with Tris-saline–5% NFDM–0.05% Nonidet P-40 and then reacted with a 1:500 dilution of peroxidase-labeled strepavidin (Kirkegaard and Perry) for 30 min at 37°C. After four additional washes, the filter was developed in 4-chloro-1-naphthol (0.6 mg/ml in phosphate-buffered saline with 20% methanol) and 0.06% hydrogen peroxide. Positive colonies were identified by a purple color.

SDS-PAGE and Western immunoblotting. SDS-polyacrylamide gel electrophoresis (PAGE) was performed essentially as described by Laemmli (23). Samples for electrophoresis were prepared by washing the cells twice with phosphate-buffered saline, sonicating for 5 min, and heating for 3 min in boiling water with sample buffer (0.06 M Tris-HCl [pH 6.8], 3% SDS, 5% mercaptoethanol, 10% glycerol, 0.01% bromophenol blue). Fifty micrograms of whole-cell lysate protein, as determined by the Bradford protein assay, was loaded in each lane of 7.5 or 10.0% acrylamide gel (8). Vertical electrophoresis was carried out at 25 mA for 4 to 6 h.

Proteins separated in gels were transferred by semidry electrophoresis onto nitrocellulose membranes (0.45-μm pore size; Bio-Rad Laboratories, Richmond, Calif.) with a Polyblot transfer system (American Bionetics, Hayward, Calif.) in a discontinuous transfer buffer as described by the manufacturer. The transfer was carried out at 450 mA for 30 min. The filters were treated with primary antibody, biotin-labeled anti-IgG (heavy and light chains), strepavidin horseradish peroxidase, and 4-chloro-1-naphthol as described above. Biotin-labeled anti-IgG (heavy and light chain) was used at a dilution of 1:250 in Tris-saline–5% NFDM–0.05% Nonidet P-40.

Immunological reagents. Canine and equine sera with IFA titers of ≥1,280 and ≥320, respectively, were obtained from animals that had signs of Lyme borreliosis, originated from an endemic area of Lyme borreliosis, and had a history of exposure to *I. dammini* ticks. Caprine hyperimmunized serum to *B. burgdorferi* was kindly provided by Kirkegaard and Perry Laboratories. Sera from humans from Minnesota and western Wisconsin with Lyme borreliosis (erythema migrans symptoms and involvement at least one other organ and an optical density [OD] reading of ≥1.00 as determined by enzyme-linked immunoabsorbent assay [ELISA]) were obtained from R. C. Johnson. Normal human, dog, and horse sera were obtained from individuals with no signs of Lyme borreliosis or history of exposure to *I. dammini* and an IFA titer of ≤32 (or an ELISA OD of ≤0.25). Mouse monoclonal antibody directed against β-galactosidase was purchased from Sigma.

Mouse antiserum directed against the 110-kDa protein of the recombinant *E. coli* was prepared as follows. The 110-kDa protein band was excised from a Coomassie-blue stained SDS gel. The excised gel slice was frozen with liquid nitrogen and crushed with a mortar and pestle. Then 5 to 10 μg of protein in acrylamide was injected intraperitoneally. Three weeks later, the mice were reinjected with 5 to 10 μg of protein subcutaneously. Two weeks after the second immunization, blood was collected by cardiac puncture and the serum was stored at −20°C.

RESULTS

Library construction and screening. The strategy for constructing a recombinant library containing *B. burgdorferi* DNA fragments was to generate an *Eco*RI partial restriction digest and collect fragments 2 to 8 kb in size by sucrose gradient centrifugation. By selecting large DNA fragments we obtained a larger proportion of clones that expressed *B. burgdorferi* proteins. *Eco*RI-digested *B. burgdorferi* DNA (90 ng) was ligated into dephosphorylated pWR590 (10 ng) and used to transform *E. coli* DH5α. Approximately 10,000 ampicillin-resistant colonies were obtained. An initial immunological screening of the library with antibodies from equal volumes of pooled sera from 5 dogs (with a titer of ≥1,280 as determined by IFA to *B. burgdorferi*) revealed 200 positive clones. In a secondary screening, 20 clones reacted with the antibodies from the pooled canine sera. This reduction was due to the inability of clones to grow on media during the isolation procedure.

Expression of the 110-kDa antigen by *E. coli*. *B. burgdorferi* antigens expressed by recombinant clones were characterized by immunoblotting with high-titered sera from dogs with Lyme borreliosis and serum from goats hyperimmunized with *B. burgdorferi*. One clone (A-1-24) expressed

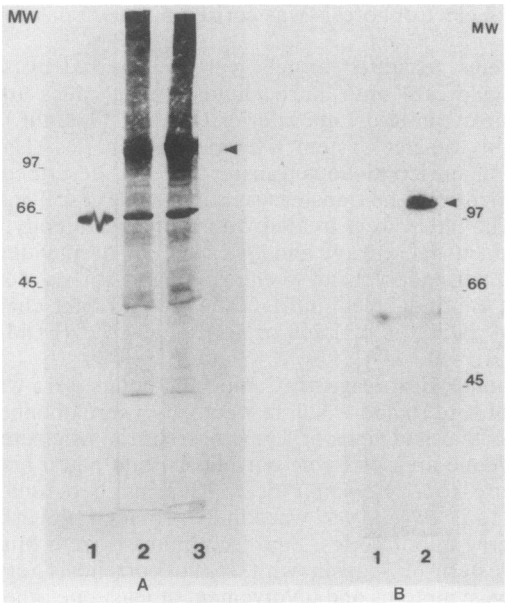

FIG. 1. Expression of the 110-kDa recombinant antigen. (A) Immunoblot of *E. coli* harboring pWR590 alone (lane 1), noninduced *E. coli* clone A-1-24 (lane 2), and *E. coli* clone A-1-24 induced with IPTG (lane 3) probed with pooled canine sera with high anti-*B. burgdorferi* titers; the sera were from dogs with clinical signs of Lyme borreliosis. (B) Immunoblot of *E. coli* harboring pWR590 (lane 1) and *E. coli* clone A-1-24 (lane 2) probed with sera from goats immunized with *B. burgdorferi* 297. Proteins separated by a 7.5% acrylamide gel were transferred to nitrocellulose and reacted with a 1:100 dilution of the immune sera. Blots were incubated with biotin-labeled anti-dog (or anti-goat) IgG and then with strepavidin horseradish peroxidase. The position of the protein migrating at 110 kDa is shown by the arrowhead. The molecular masses of proteins are expressed in kilodaltons.

a 110-kDa antigen that reacted strongly with both sera (Fig. 1A and B). A control *E. coli* clone bearing an unrelated DNA insert in the same vector did not express the 110-kDa antigen (Fig. 1). The 110-kDa protein was expressed with and without IPTG. This observation indicated the 110-kDa protein was not expressed as a fusion protein with β-galactosidase under the control of *lacZ*. Verification of this was confirmed by colony hybridization with a monoclonal antibody to β-galactosidase, which only reacted with the clone after stimulation with IPTG (Fig. 2). It appeared that *E. coli* was using a *Borrelia* promoter to produce the 110-kDa protein.

Identification of the equivalent antigen in *B. burgdorferi* and cross-reaction of the 110-kDa protein with antigens found in other spirochetes. To identify the *B. burgdorferi* protein that corresponded to the cloned antigen, monospecific antisera to the recombinant 110-kDa protein were raised in mice. The mouse antiserum to the 110-kDa protein reacted with the recombinant 110-kDa protein in *E. coli* clone A-1-24 but not with *E. coli* harboring pWR590 alone (Fig. 3). Sera from mice immunized with acrylamide excised from the 110-kDa location in a lane containing *E. coli*(pWR590) lysate did not react with the 110-kDa recombinant protein. Surprisingly, the antiserum to the 110-kDa protein recognized a protein of 75 kDa in *B. burgdorferi* lysates (Fig. 3). Since the recombinant protein does not appear to be a fusion product, the reason for the observed difference in SDS-PAGE mobility is not known.

Mouse antiserum to the 110-kDa protein was used to determine whether cross-reactions might exist between the recombinant 110-kDa protein and an antigen(s) found in other spirochetes (Fig. 3). Antiserum to the 110-kDa protein reacted weakly with a 75-kDa protein of *B. anserina* and *B. hermsii* and with 70- and 65-kDa proteins of *Leptospira* spp.

The band intensity of the antisera to the 110-kDa proteins was 10-fold less than that of antibodies to the 75-kDa *B. burgdorferi* protein in lanes containing equivalent amounts of bacterial lysates. Since the DNA insert encoding the 110-kDa protein had no homology with other spirochetes, as

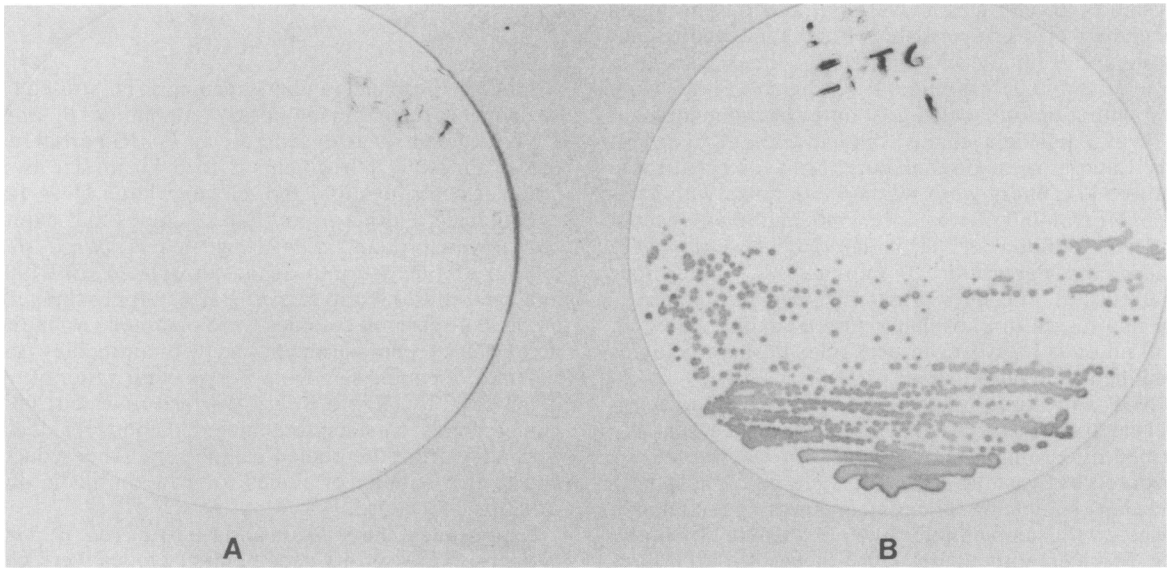

FIG. 2. Induction of *E. coli* clone A-1-24 with IPTG. Colony blot of noninduced *E. coli* clone A-1-24 (A) and *E. coli* clone A-1-24 induced with IPTG (B) probed with polyclonal antibody to β-galactosidase. Lysed colonies were reacted with a 1:100 dilution of the immune sera. The blots were incubated with biotin-labeled anti-mouse IgG and then with strepavidin horseradish peroxidase.

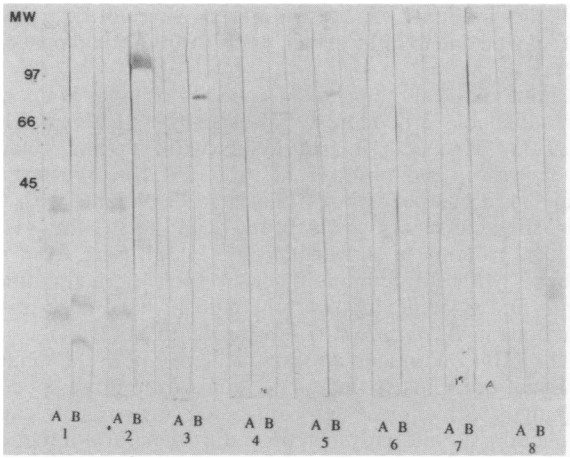

FIG. 3. Identification of the equivalent antigen in *B. burgdorferi* and cross-reaction of antiserum to the 110-kDa protein to antigens in other spirochetes. Immunoblot of *E. coli* harboring pWR590 alone (lanes 1), *E. coli* clone A-1-24 (lanes 2), *B. burgdorferi* (lanes 3), *B. anserina* (lanes 4), *B. hermsii* (lanes 5), and *L. interrogans* serovar canicola (lanes 8) with pooled serum from mice immunized with the gel slice located at 110 kDa from *E. coli* harboring pWR590 (A) or with pooled sera from mice immunized with 110-kDa cut from a SDS-PAGE gel of clone A-1-24 (B). Proteins separated by a 10.0% acrylamide gel were transferred to nitrocellulose and reacted with a 1:100 dilution of the immune serum. The blot was incubated with biotin-labeled anti-mouse IgG and then with strepavidin horseradish peroxidase. The molecular masses of proteins are expressed in kilodaltons.

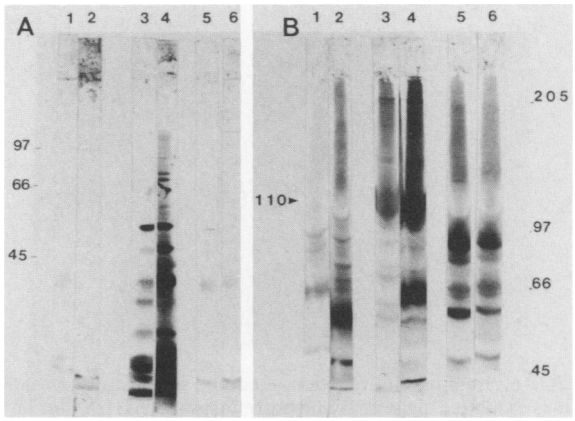

FIG. 4. Immunological reaction of *E. coli* clone A-1-24 with sera from humans with Lyme borreliosis. Immunoblots of *B. burgdorferi* MM1 (A) and *E. coli* clone A-1-24 (B) probed with negative sera (OD ≤0.25) from patients with no symptoms of Lyme borreliosis (lanes 1 and 2), or high-titered sera from individuals (OD ≥1.0 as determined by ELISA) with clinical symptoms of Lyme borreliosis (lanes 3 through 6). Proteins separated on a 10% (A) or 7.5% (B) acrylamide gel were transferred to nitrocellulose and reacted with a 1:100 dilution of the immune sera. The blot was incubated with biotin-labeled anti-human IgG and then with strepavidin horseradish peroxidase. The position of the protein migrating at 110 kDa is shown by the arrowhead. The molecular masses of proteins are expressed in kilodaltons.

determined by Southern blotting (unpublished data), we presumed that the cross-reacting proteins were unrelated.

Immunological reactions of *E. coli* clone A-1-24 with sera from humans with Lyme borreliosis. In the immunoblot assay, the 110-kDa antigen from clone A-1-24 reacted with four of seven sera from patients with Lyme borreliosis as diagnosed from erythema migrans lesions and a positive reaction with the ELISA (Table 1). The sera that reacted with the 110-kDa antigen (Fig. 4B, lanes 3 and 4) also reacted with the 31-, 34-, and 41-kDa antigens of *B. burgdorferi* on an immunoblot (Fig. 4A, lanes 3 and 4). The sera that did not react with the 110-kDa protein (Fig. 4B, lanes 5 and 6) all reacted with the 41-kDa antigen (Fig. 4A, lanes 5 and 6), the protein recognized early in the immune response (2, 39). Sera from humans with no clinical symptoms of Lyme borreliosis and an OD of ≤0.25 in the ELISA did not react with the 110-kDa antigen (Fig. 4B; lanes 1 and 2) or the whole-cell lysate of *B. burgdorferi* (Fig. 4A, lanes 1 and 2).

TABLE 1. Comparison of immunoblotting reactions with the *E. coli* clone expressing the 110-kDa antigen to the *B. burgdorferi* lysate and sera from human and animals

Serum source	No. with the following reaction with *B. burgdorferi* lysate/total[a]	
	Positive	Negative
Human	4/7	0/9
Dog	10/10	0/8
Horse	5/6	0/11

[a] Ratios represent the numbers of serum samples that reacted with the 110-kDa protein/the total numbers of samples tested. A reaction was scored as positive if the serum reacted with two or more antigens of *B. burgdorferi*.

One of these sera reacted with the 41-kDa antigen of *B. burgdorferi* and may be a result of a recent spirochete infection (2, 15).

Immunological reactions of *E. coli* clone A-1-24 with sera from domestic animals with Lyme borreliosis. In the immunoblot assay, the 110-kDa antigen from clone A-1-24 reacted with all sera from dogs with lameness and clinical signs of Lyme borreliosis (Table 1). These sera, which had a high

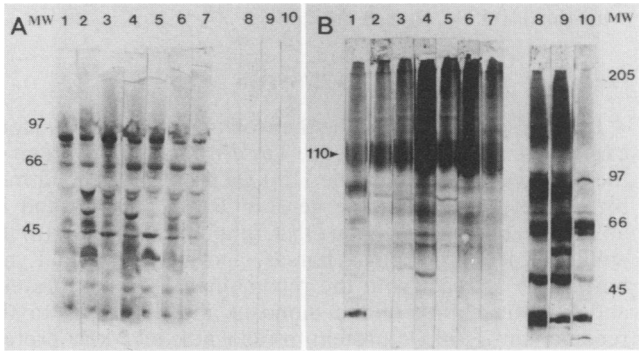

FIG. 5. Immunological reaction of *E. coli* clone A-1-24 with sera from dogs with Lyme borreliosis. Immunoblots of *B. burgdorferi* MM1 (A) and *E. coli* clone A-1-24 (B) probed with high-titered sera (IFA titer of ≥1,280) from individual animals with clinical symptoms of Lyme borreliosis (lanes 1 through 7), or negative sera (IFA titer of ≤32) from animals with no symptoms of Lyme borreliosis (lanes 8 through 10). Proteins separated on a 10% (A) or 7.5% (B) acrylamide gel were transferred to nitrocellulose and reacted with a 1:100 dilution of the immune sera. The blot was incubated with biotin-labeled anti-dog IgG and then with strepavidin horseradish peroxidase. The position of the protein migrating at 110 kDa is shown by the arrowhead. The molecular masses of proteins are expressed in kilodaltons.

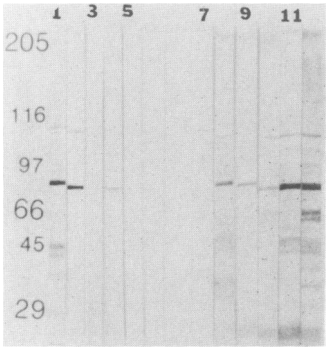

FIG. 6. Immunoblot reaction of equine sera with *B. burgdorferi*. Horses exhibited signs of Lyme borreliosis and an IFA titer of >320. Fifty micrograms of lysed *B. burgdorferi* protein was loaded in each lane, subjected to SDS-PAGE (7.5% polyacrylamide gel), and electroblotted onto nitrocellulose paper. The nitrocellulose paper was cut into strips and incubated with a 1:100 dilution of equine serum; then the strips were incubated with peroxidase-labeled anti-horse IgG (heavy and light chains). Lanes 1 through 12 show different patterns depending on the stage in the disease process. Lanes 3, 5, and 6 exhibited very faint bands, approximately 10-fold fainter than those in lane 4. Lane 7 was negative for any reaction with the antigen.

IFA titer (≥1,280), reacted with the 31-, 34-, 41-, and 75-kDa antigens of *B. burgdorferi* (Fig. 5A, lanes 1 through 7) and the 110-kDa antigen of the *E. coli* clone (Fig. 5B, lanes 1 through 7). Sera from dogs with no clinical signs of Lyme borreliosis and a IFA titer of ≤32 were nonreactive with the 110-kDa antigen (Fig. 5B, lanes 8 through 10) and did not react with any antigen of *B. burgdorferi* (Fig. 5A, lanes 8 through 10).

The 110-kDa antigen from clone A-1-24 reacted on immunoblotting with five of six sera from horses with clinical signs of Lyme borreliosis (Table 1). These sera had IFA titers of ≥320 and positive reactions to *B. burgdorferi* on immunoblots (Fig. 6). Sera from horses with no clinical signs of Lyme borreliosis and an IFA titer of ≤32 were nonreactive with the 110-kDa cloned antigen.

DISCUSSION

In this report, we describe the isolation of an *E. coli* clone expressing a 110-kDa antigen. The *B. burgdorferi* origin of the cloned gene encoding the 110-kDa protein was confirmed by (i) the presence of the gene in *B. burgdorferi* and its absence in other spirochetal DNA (unpublished data), (ii) the ability of sera from dogs, horses, and humans with Lyme borreliosis to react with the recombinant 110-kDa protein, and (iii) the ability of the monospecific antiserum to the recombinant 110-kDa protein to recognize a 75-kDa protein of *B. burgdorferi*.

Evidence that the 110-kDa antigen of the *E. coli* clone A-1-24 is equivalent to the 75-kDa antigen of *B. burgdorferi* includes (i) the ability of the monospecific antiserum to the recombinant 110-kDa protein to recognize a 75-kDa protein of *B. burgdorferi* and (ii) the observation that the majority of the positive sera from individuals with Lyme borreliosis that reacted with the 110-kDa antigen in *E. coli* clone A-1-24 also reacted with the 75-kDa antigen of *B. burgdorferi*. A possible explanation of why sera from individuals with Lyme borreliosis reacted with the 110-kDa protein and not the 75-kDa antigen is that the 110-kDa recombinant protein is produced in higher amounts. The size difference cannot be explained but may be due to differences in post translational processing (12).

Immunological diagnosis of Lyme borreliosis is uncertain with available procedures. False-positive IFA and ELISA reactions can occur in individuals with syphilis, relapsing fever, yaws, and pinta (2, 21, 27, 29). By Western blot analysis, it has been shown that sera of patients with these spirochetal diseases show cross-reactions to the 41- and 60-kDa proteins of *B. burgdorferi* (2, 16). Sera from some patients with autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus have false-positive reactions in *B. burgdorferi* serological tests (2, 34).

The 110-kDa antigen appears to be useful in the diagnosis of Lyme borreliosis, since individuals from three species of mammals with clinical signs of Lyme borreliosis are capable of eliciting an antibody response during natural infection to the recombinant 110-kDa antigen. False-positive reactions were not observed with the 110-kDa antigen. Sera from humans, dogs, and horses with no signs of Lyme borreliosis and titers at background levels did not react with the 110-kDa antigen on immunoblots. Second, the reaction of antiserum to the 110-kDa protein with other spirochetes was minimal (i.e., ≤10%), and hybridization of the DNA insert to other *Borrelia* spp. was nonexistent (unpublished data). Further work is needed to determine when and how many individuals that have Lyme borreloisis produce antibodies that react with the 110-kDa antigen.

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REFERENCES

1. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57**:521–525.
2. **Barbour, A. G., W. Burgdorfer, E. Grunwaldt, and A. C. Steere.** 1983. Antibodies of patients with Lyme disease to components of the *Ixodes dammini* spirochete. J. Clin. Invest. **72**:504–515.
3. **Barbour, A. G., and C. F. Garon.** 1987. Linear plasmids of the bacterium *Borrelia bardorferi* have covalently closed ends. Science **237**:409–411.
4. **Barbour, A. G., S. F. Hayes, R. A. Heiland, M. E. Schrumpf, and S. L. Tessler.** 1986. A *Borrelia* genus-specific monoclonal antibody binds to a flagellar epitope. Infect. Immun. **52**:549–554.
5. **Barbour, A. G., R. A. Heiland, and T. R. Howe.** 1985. Heterogeneity of major proteins in Lyme disease *Borrelia*: a molecular analysis of American and European isolates. J. Infect. Dis. **152**:478–484.
6. **Bergstrom, S., V. G. Bundoc, and A. G. Barbour.** 1989. Molecular analysis of linear plasmid encoded major surface proteins, OspA and OspB of the Lyme disease spirochete *Borrelia burgdorferi*. Mol. Microbiol. **4**:479–486.
7. **Bey, R. F., and R. C. Johnson.** 1978. Protein-free and low-protein medium for the cultivation of *Leptospira*. Infect. Immun. **19**:562–569.
8. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–253.
9. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease, a tick-borne spirochetosis? Science **216**:1317–1319.
10. **Burgess, E. C.** 1985. Natural exposure of Wisconsin dogs to the Lyme disease spirochete (*Borrelia burgdorferi*). Lab. Anim. Sci. **36**:288–290.
11. **Burgess, E. C., D. Gillette, and J. P. Pickett.** 1986. Arthritis and panuveitis as manifestations of *Borrelia burgdorferi* infection in

a Wisconsin pony. J. Am. Vet. Med. Assoc. **189**:1340–1342.
12. **Charles, I. G., G. Dougan, D. Pickard, S. Chatfield, M. Smith, P. Morrissey, and N. F. Fairweather.** 1989. Molecular cloning and characterization of protective outer membrane protein P.69 from *Bordetella pertussis*. Proc. Natl. Acad. Sci. USA **86**:3554–3558.
13. **Collins, C., and G. Peltz.** 1991. Immunoreactive epitopes on an expressed recombinant flagellar protein of *Borrelia burgdorferi*. Infect. Immun. **59**:514–520.
14. **Dryer, R. F., P. G. Broellner, and A. S. Carney.** 1979. Lyme arthritis in Wisconsin. J. Am. Med. Assoc. **241**:498–499.
15. **Fikrig, E., S. W. Barthold, F. S. Kantor, and R. A. Flavell.** 1990. Protection of mice against the Lyme disease agent by immunizing with the recombinant OspA. Science **250**:553–556.
16. **Grodzicki, R. L., and A. C. Steere.** 1988. Diagnosing early Lyme disease by immunoblotting comparison with indirect ELISA using different antigen preparations. J. Infect. Dis. **157**:790–797.
17. **Guo, L., P. P. Stepien, J. Y. Tso, R. Brosseau, S. Narang, D. Y. Thomas, and R. Wu.** 1984. Synthesis of human insulin gene. Construction of expression vectors for fused proinsulin production in *Escherichia coli*. Gene **29**:251–254.
18. **Hansen, K., J. M. Bangsborg, H. Fjordvang, N. Strandberg Pederson, and P. Hindersson.** 1988. Immunochemical characterization of and isolation of the gene for a *Borrelia burgdorferi* immunodominant 60-kilodalton antigen common to a wide range of bacteria. Infect. Immun. **56**:2047–2053.
19. **Howe, T. R., F. W. LaQuier, and A. G. Barbour.** 1985. Organization of genes encoding two outer membrane proteins of the Lyme disease agent *Borrelia burgdorferi* within a single transcriptional unit. Infect. Immun. **54**:207–212.
20. **Howe, T. R., L. W. Mayer, and A. G. Barbour.** 1985. A single recombinant plasmid expressing two major outer surface proteins of the Lyme disease spirochete. Science **227**:645–646.
21. **Hunter, E. F., H. Russell, C. E. Farshy, J. S. Sampson, and S. A. Larsen.** 1986. Evaluation of sera from patients with Lyme disease in the fluorescent treponemal antibody-absorption test for syphilis. Sex. Transm. Dis. **13**:232–236.
22. **Kornblatt, A. N., P. H. Urband, and A. C. Steere.** 1985. Arthritis caused by *Borrelia burgdorferi* in dogs. J. Am. Vet. Med. Assoc. **186**:960–964.
23. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
24. **Lawson, J. P., and A. C. Steere.** 1985. Roentgenologic findings in Lyme arthritis. Radiology **154**:37–43.
25. **Lissman, B. A., E. M. Bosler, H. Camay, B. G. Ormiston, and J. L. Benach.** 1984. Spirochete-associated arthritis (Lyme disease) in a dog. J. Am. Vet. Med. Assoc. **185**:219–220.
26. **Loken, K. I., C. C. Wu, R. C. Johnson, and R. F. Bey.** 1985. Isolation of the Lyme disease spirochete from mammals in Minnesota. Proc. Soc. Exp. Biol. Med. **179**:300–302.
27. **Magnarelli, L. A., J. F. Anderson, and R. C. Johnson.** 1987. Cross-reactivity in serological tests for Lyme disease and other spirochetal infections. J. Infect. Dis. **156**:183–188.
28. **Magnarelli, L. A., J. F. Anderson, A. B. Schreier, and C. M. Ficke.** 1987. Clinical and serological studies of canine borreliosis. Am. J. Vet. Assoc. **191**:1089–1094.
29. **Magnarelli, L. A., J. N. Miller, J. F. Anderson, and G. R. Riviere.** 1990. Cross-reactivity of nonspecific treponemal antibody in serologic tests for Lyme disease. J. Clin. Microbiol. **28**:1276–1279.
30. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Marcus, L. C., M. M. Patterson, R. E. Gillfillan, and P. H. Urband.** 1986. Antibodies to *Borrelia burgdorferi* in New England horses. Am. J. Vet. Res. **46**:2570–2571.
32. **Pachner, A. R., and A. C. Steere.** 1985. The triad of neurologic abnormalities of Lyme disease: meningitis, cranial neuritis, and radiculoneuritis. Neurology **35**:47–53.
33. **Reik, L. A., A. C. Steere, N. H. Bartenhagen, R. E. Shope, and S. E. Malawista.** 1979. Neurological abnormalities of Lyme disease. Medicine **58**:281–294.
34. **Russell, H., J. S. Sampson, G. F. Schmidt, H. W. Wilkinson, and B. Plikaytis.** 1984. Enzyme-linked immunosorbent assay and indirect immunofluorescence assay for Lyme disease. J. Infect. Dis. **149**:465–470.
35. **Smibert, R. M.** 1976. Cultivation, composition, and physiology of avirulent treponemes, p. 49–56. *In* R. C. Johnson (ed.), The biology of parasitic spirochetes. Academic Press, Inc., New York.
36. **Steere, A. C., N. H. Bartenhagen, J. E. Craft, G. J. Hutchinson, D. W. Rahn, L. H. Sigal, P. N. Spieler, K. S. Stenn, and S. E. Malawista.** 1983. The early clinical manifestation of Lyme disease. Ann. Intern. Med. **93**:8–16.
37. **Steere, A. C., W. P. Batsford, M. Weinberg, J. Aexander, H. J. Berger, S. Wolfson, and S. E. Malawista.** 1980. Lyme carditis: cardiac abnormalities of Lyme disease. Ann. Intern. Med. **93**:8–16.
38. **Steere, A. C., C. E. Brinckerhoff, D. J. Miller, H. Drinker, E. D. Harris, and S. E. Malawista.** 1980. Elevated levels of collagenase and prostaglandin E2 from synovium associated with erosion of cartilage and bone in a patient with chronic Lyme arthritis. Arthritis Rheum. **23**:591–599.
39. **Steere, A. C., A. Gibofsky, M. E. Patarroyo, R. J. Winchester, J. A. Hardin, and S. E. Malawista.** 1979. Chronic Lyme arthritis: clinical and immunogenetic differentiation from rheumatoid arthritis. Ann. Intern. Med. **90**:896–901.
40. **Stoenner, H. G.** 1976. Treatment and control of leptospirosis, p. 375–388. *In* R. C. Johnson (ed.), The biology of parasitic spirochetes. Academic Press, Inc., New York.
41. **Wallich, R., S. E. Moter, M. M. Simon, K. Ebnet, A. Heiberger, and M. D. Kramer.** 1990. The *Borrelia burgdorferi* flagellum-associated 41-kilodalton antigen (flagellin): molecular cloning, expression and amplification of the gene. Infect. Immun. **58**: 1711–1718.
42. **Young, R. A., and R. W. Davis.** 1983. Efficient isolation of genes by using antibody probes. Proc. Natl. Acad. Sci. USA **80**:1194–1198.