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A Single Recombinant Plasmid Expressing Two Major Outer Surface Proteins of the Lyme Disease Spirochete

Abstract. *A gene bank of DNA from the Lyme disease spirochete was constructed in the plasmid pBR322. Plasmid pTRH32, a recombinant that in* Escherichia coli *expresses the two major outer surface proteins of the Lyme disease spirochete, was identified. One of the recombinant products, designated OspA, represents a surface protein that appears to be common to all Lyme disease spirochetes, whereas the other recombinant product, designated OspB, represents a more variable surface protein. This recombinant plasmid provides a foundation for future studies on the epidemiology and pathogenesis of Lyme disease as well as on the genetic organization of the etiologic agent.*

Lyme disease is a tick-borne disorder characterized by a distinctive skin lesion (erythema chronicum migrans), meningoradiculitis, cardiac abnormalities, and an arthritis (*1*). The etiologic agent of this disorder, a hitherto unknown spirochete (*2*), has been designated *Borrelia burgdorferi* (*3*). This organism has been isolated from the blood, skin, and cerebrospinal fluid of infected patients (*4, 5*), from the tick vectors *Ixodes dammini* (*2, 4*), *I. ricinus* (*6*), and *Amblyomma americanum* (*7*), and from mammals in areas in which the disease is endemic (*8, 9*).

The chronicity of some of the manifestations of Lyme disease, such as the oligoarticular arthritis and meningoradiculitis, suggests that the host cannot effectively rid itself of the infecting agent. Alternatively, the host's immune response to the spirochete may actually induce or accentuate the pathological lesions associated with this disorder. Thus, knowledge of the balance struck between the host's immune system and the spirochete during chronic infection may be a key to understanding the pathogenesis of Lyme disease.

In earlier studies, we identified several antigenic components of the spirochete causing Lyme disease (LD spirochete) by their reactivities with antibodies in the sera of patients with the disease (*10, 11*). One of these components, a surface protein with an apparent molecular weight of 31,000 (31K), was common to all LD spirochetes examined (*12*). More recently, variations were shown to occur in the antigenic determinants and apparent molecular weight of another major outer surface protein (*13*). The apparent molecular weight of this latter protein was approximately 34K (*13*). We now designate the 31K and 34K surface proteins as OspA (outer surface protein A) and OspB, respectively.

Our approach toward understanding the pathogenesis and immunology of Lyme disease and related disorders initially entailed the cloning of LD spirochete DNA and the identification of recombinant clones that express LD spirochete antigens. This approach facilitates the production of individual LD spirochete antigens free of contaminating LD spirochete proteins and animal serum components. We now report the construction of a gene bank of DNA from the LD spirochete in *Escherichia coli* and the isolation of a recombinant clone that expresses two of the major surface antigens of the spirochete.

The LD spirochete strain B31 (A.T.C.C. 35210) was initially isolated from the tick vector *I. dammini* (*2*). This organism was grown in BSK II medium as described (*13*). Chromosomal DNA was obtained (*14*) and partially digested with the restriction enzyme Sau 3AI (Bethesda Research Laboratories); 1.5- to 8-kb (kilobase) fragments were isolated after the DNA was subjected to electrophoresis in low–melting temperature agarose (Seaplaque, FMC Corporation) (*15*). These fragments were ligated into the Bam HI site of dephosphorylated pBR322 (New England Biological Laboratories) with T4 DNA ligase (Bethesda Research Laboratories) by using a 3:1 ratio (by weight) of chromosomal to vector DNA. *Escherichia coli* strain ED8654 (*met gal supE supF hsdR*) was transformed with the ligated mixture as described (*16*). Ampicillin-resistant colonies were obtained and subsequently screened in situ by colony radioimmunoassay (*17*). Our source of antibody reactive with LD spirochete components was synovial fluid from a patient with chronic Lyme arthritis. This synovial fluid has been shown to have a titer of 1:2560 for LD spirochetes in an immunofluorescence assay and to react with several LD spirochete components (*11*). The specificity of this antiserum was enhanced further by adsorption with a sonicate of *E. coli* ED8654 (pBR322) before assay.

Approximately 10,000 colonies were examined by the colony blot assay, and at least three colonies were identified that produced detectable signals in the autoradiographs. Whole cells of these recombinants were lysed with sodium dodecyl sulfate (SDS), and components of the lysates were electrophoretically separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose for Western blot analysis as described (*11, 12*). This analysis confirmed the expression of LD spirochete antigens by recombinants identified in the colony blot assay. One recombinant (pTRH32) was identified that expressed two immunoreactive pro-

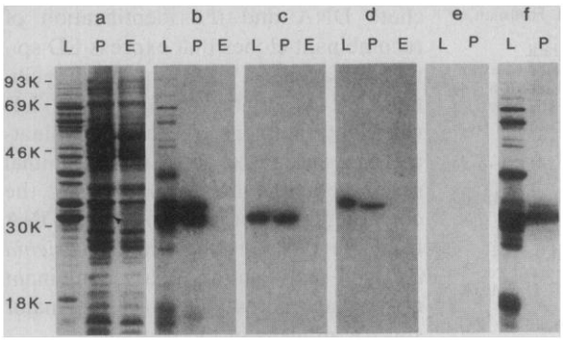

Fig. 1. Western blot analyses of proteins encoded by pTRH32. Sodium dodecyl sulfate lysates of LD spirochete B31 (L), *E. coli* ED8654 containing pTRH32 (P), and *E. coli* ED8654 containing pBR322 (E) were subjected to SDS-PAGE (12.5 percent acrylamide concentration). (a) Coomassie blue–stained proteins in the three lysates. The arrowhead indicates a protein that was detectable in P lysate but not in E (the *E. coli* and plasmid control). (b to f) The reactivities in blots of antibodies from (b) synovial fluid from a patient with chronic Lyme arthritis (*12*); (c) H5332, a monoclonal antibody to the OspA (31K) protein of LD spirochete B31 (*13*); (d) H6831, a monoclonal antibody to the OspB (34K) protein of LD spirochete B31 (*14*); (e) serum from patient H during the initial acute episode of erythema chronicum migrans [serum H1 (*10*)]; and (f) serum from patient H during convalescence from Lyme arthritis [serum H3 (*10*)]. The procedure for Western blot analysis has been described (*18*); bound antibody was detected with ^{125}I-labeled protein A. The hybridoma supernatants (H5332 and H6831) were diluted 1:10, and the synovial fluid and the two sera from patient H were diluted 1:100. The relative migrations of the standards are given as the molecular weights × 1000 (K).

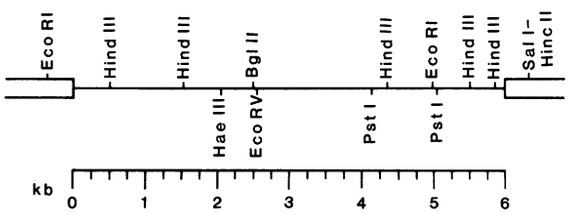

Fig. 2. Partial restriction endonuclease map of the LD spirochete DNA of recombinant pTRH32. The 6-kb insert is circumscribed by Sau 3AI or Bam HI sites at the insert vector junctions. Several restriction enzymes did not cut the LD spirochete DNA of this recombinant, including Ava I, Bam HI, Bgl I, Bss HI, Cla I, Hinc II, Mlu I, Mst II, Nru I, Pvu I, Sal I, Sph I, Sst I, Sst II, Xho I, and Xma III. Initial results of *Tn5* insertion mutagenesis suggest that the genes encoding OspA and OspB are both located within the last 2.5 kb of the cloned LD spirochete DNA (*19*).

teins with apparent molecular weights of approximately 31K and 34K, respectively (Fig. 1).

Because these two proteins migrated at rates approximately equal to those of the two major LD spirochete surface proteins, whole cell lysates of this recombinant were examined by Western blot analysis with monoclonal antibodies H5332 and H6831, which react with the OspA and OspB proteins, respectively, of strain B31 (*12, 13*). This confirmed that the recombinant was expressing the OspA and OspB proteins of the LD spirochete (Fig. 1). Monoclonal antibody H5TS, which binds to OspB proteins of some other strains but not to that of strain B31 (*13*), was nonreactive in the Western blot analysis. An association between the expressed antigens and Lyme disease was shown further by the presence of reactive antibodies in serum from a patient in remission from Lyme arthritis but not in serum from the same patient in the acute stage of the disease (Fig. 1). We do not know the basis for the slight differences in mobility of the OspA and OspB proteins of the recombinant and the LD spirochete. Nevertheless, the results of these analysis indicate that we have cloned the genes that encode the OspA and OspB surface proteins of the strain B31 spirochete.

Plasmid DNA from the recombinant encoding the two LD spirochete surface antigens was prepared by alkaline lysis and purified by ethidium bromide–cesium chloride density gradient centrifugation (*18*). A partial restriction endonuclease map of this recombinant, pTRH32, was then constructed (Fig. 2).

Our findings indicate that at least some LD spirochete genes may be stably maintained and expressed in *E. coli*. Whether the OspA and OspB proteins will be effective in the prevention of LD spirochete infection through active immunization remains to be determined. The isolation in *E. coli* of the genes coding for these surface proteins should, nevertheless, make such a determination of efficacy easier and potentially safer than fractionation of whole spirochetes. A more immediate use of the recombinant and derivative subclones will be for the examination of the gene arrangements of other LD spirochete strains, particularly in regard to the more variable OspB proteins, and for use as a DNA hybridization probe for specimens from patients with Lyme disease.

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