

Characterization of *Borrelia burgdorferi* Proteins Reactive with Antibodies in Synovial Fluid of a Patient with Lyme Arthritis

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Four *Borrelia burgdorferi* proteins reactive with antibodies in the synovial fluid of a patient with Lyme arthritis were characterized. Homology between amino acid sequences of immunoreactive spirochetal proteins and human proteins, including members of the *Escherichia coli* GroEL protein family, suggests that antigenic mimicry may play a role in the pathogenesis of Lyme arthritis.

It is hypothesized that inflammation that produces organ damage in Lyme disease is initiated, and possibly perpetuated, by the immune response to certain proteins of the causative agent, *Borrelia burgdorferi* (4, 7). Therefore, important insight into the immunopathogenesis of Lyme arthritis should be obtained from the characterization of *Borrelia* proteins which induce B-cell and T-cell responses in patients with Lyme arthritis. Previous studies have demonstrated that antibodies reactive with spirochetal proteins are present in the sera of patients with Lyme disease (2). In the early stages of Lyme disease, the antibodies are primarily reactive with a 41-kilodalton (kDa) *Borrelia* protein, while in the chronic stage, patients develop antibodies to as many as 11 different spirochetal proteins (5). In this paper, we present the characterization, by NH₂-terminal sequence analysis, of *Borrelia* proteins reactive with antibodies in the synovial fluid of a patient with chronic Lyme arthritis.

Protein lysates of the CA12 strain of *B. burgdorferi* were prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (1; H. Yssel et al., submitted for publication). Synovial fluid, obtained from the knee of a patient with chronic Lyme arthritis (Yssel et al., submitted), was preabsorbed for 2 h with a lysate of *Escherichia coli* JM109 prior to use in immunoblot analysis.

One-dimensional (1D) and separation in the second dimension of 2D SDS-PAGE gels was performed by using gels containing 10% acrylamide under reducing conditions. 2D gels were run as described by O'Farrell (17) by using Ampholines with a pH range of 3.5 to 10.0 (Pharmacia-LKB, Piscataway, N.J.).

Proteins separated by 1D or 2D gel electrophoresis were transferred onto nitrocellulose for immunoblotting (23). The blocking procedure, as well as incubation with a 1/50 dilution of preabsorbed synovial fluid, and detection of bound antibody by using alkaline phosphatase-conjugated goat anti-human immunoglobulin G (Bio-Rad Laboratories, Richmond, Calif.) were performed according to the instructions of the manufacturer.

Spirochetal proteins separated by 1D (41- and 58-kDa proteins) and 2D (46- and 60-kDa proteins) gel electrophoresis were electroblotted onto a PVDF membrane (16). Amino-terminal sequences were determined by using a gas-phase sequenator (model 470A; Applied Biosystems, Foster City,

Calif.) with initial yields of 30, 10, 100, and 10 pmol for the 41-, 46-, 58-, and 60-kDa proteins, respectively. Sequence analysis of two separate preparations of each of the immunoreactive proteins yielded identical results. Amino acid sequence homology was determined by searching the SWISS-PROT11 data base by using PC/GENE (Intelligenetics, Mountain View, Calif.).

Immunoblot analysis demonstrated that four *Borrelia* proteins of 41, 46, 58, and 60 kDa (relative molecular mass) were consistently reactive with antibodies in the synovial fluid of this patient with Lyme arthritis (Fig. 1 and 2). Serum samples obtained from 12 other patients with Lyme arthritis, but not from four healthy controls, had antibody reactivity with the 41-, 58-, and 60-kDa spirochete proteins. Only two other patients with Lyme arthritis had antibodies reactive with the 46-kDa protein (data not shown). The synovial fluid antibodies reacted with these four *B. burgdorferi* proteins after purification but not with other purified *Borrelia* proteins, including the OspA protein (Fig. 2). The 46- and 60-kDa proteins were reproducibly more intensely stained than the 41- and 58-kDa *B. burgdorferi* proteins.

The NH₂-terminal amino acid sequences of the four immunoreactive *B. burgdorferi* proteins are presented in Fig. 3. Of 10 NH₂-terminal amino acids of the 41-kDa *Borrelia* protein, 8 are identical to those of the *Treponema pallidum* endoflagellin (3, 15; Fig. 3, bottom). The NH₂-terminal amino acid sequence of the immunoreactive 46-kDa *B. burgdorferi* protein has homology to the heavy chain of chicken smooth muscle (amino acids 468 to 480) (26) and human skeletal muscle (amino acids 465 to 477) (8) myosins (Fig. 3, bottom). Alignment of the amino acids at these positions in the proteins reveals that six positions are identical and three positions have highly conservative substitutions (Tyr → Phe, Val → Leu, and Ile → Leu).

The amino acid sequence of the 60-kDa *B. burgdorferi* protein did not have significant sequence homology with any other protein in the data base. However, the amino-terminal sequence of the 58-kDa *Borrelia* protein has significant sequence homology with that of the *E. coli* GroEL protein (11) (7 of 11 consecutive amino acids in the two protein sequences are identical) and with those of other proteins in the GroEL family, including the 65-kDa major antigen of mycobacterium (22) and the human P1 mitochondrial protein (12; Fig. 3, bottom). This sequence homology is consistent with data from Hansen et al. that show that a 60-kDa spirochetal protein cross-reacts with antibodies directed

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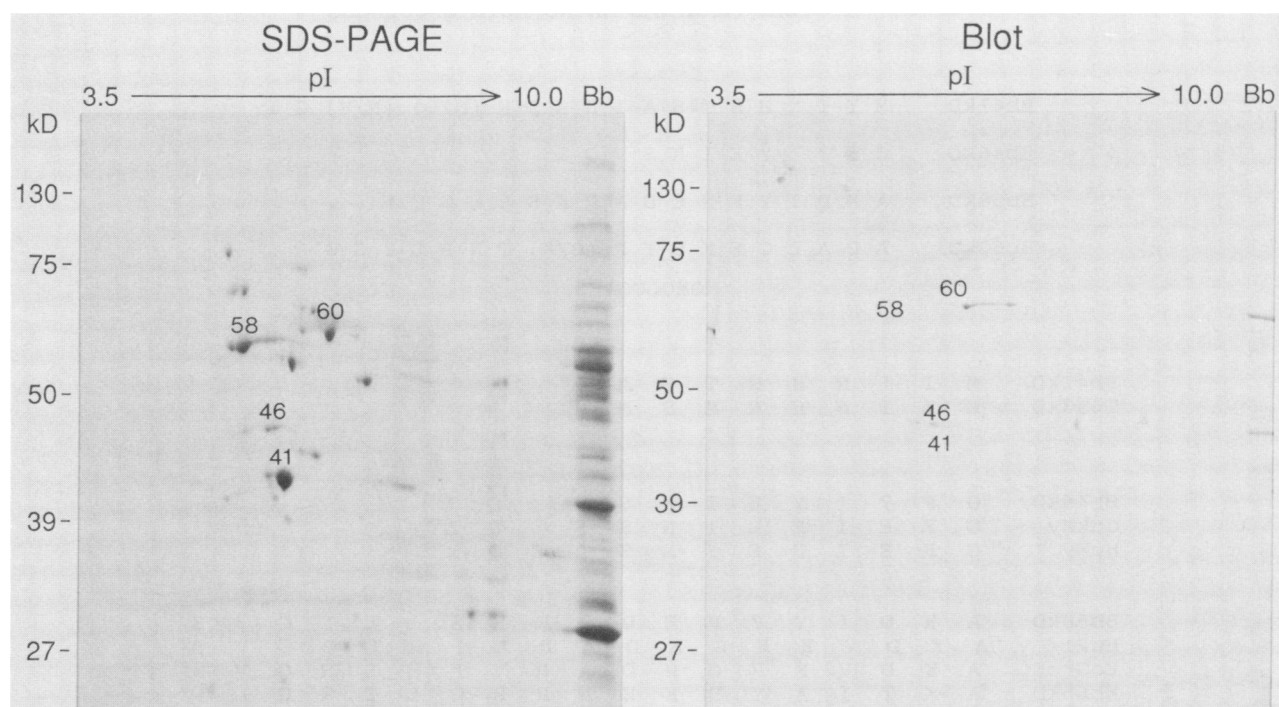


FIG. 1. Coomassie blue-stained 2D isoelectric focusing-SDS-PAGE of *B. burgdorferi* proteins and immunoblot using synovial fluid from a patient with late Lyme arthritis. Separation of proteins in 1D was by isoelectric focusing between pH 3.5 and 10.0; separation in 2D was by SDS-10% PAGE. The relative molecular masses of four proteins recognized by synovial fluid antibodies are indicated above each protein. A *Borrelia* lysate separated by 1D SDS-PAGE (Bb) is also shown.

against GroEL proteins of other bacteria (10). However, another immunoreactive spirochete protein of similar molecular mass (60 kDa) was not a GroEL homolog.

There are at least two distinct mechanisms through which an infectious microorganism can produce chronic inflammation in host tissue. One possibility is that the infecting organism or a nondegradable component of the organism

remains within the target organ tissue and continually elicits an inflammatory response. During the chronic phase of Lyme arthritis, the patients have detectable spirochetes in their synovia (13) and develop additional antibodies of both immunoglobulin M and immunoglobulin G isotypes specific for new *Borrelia* antigens (5). This suggests that viable spirochetes or spirochete antigens persist in these patients.

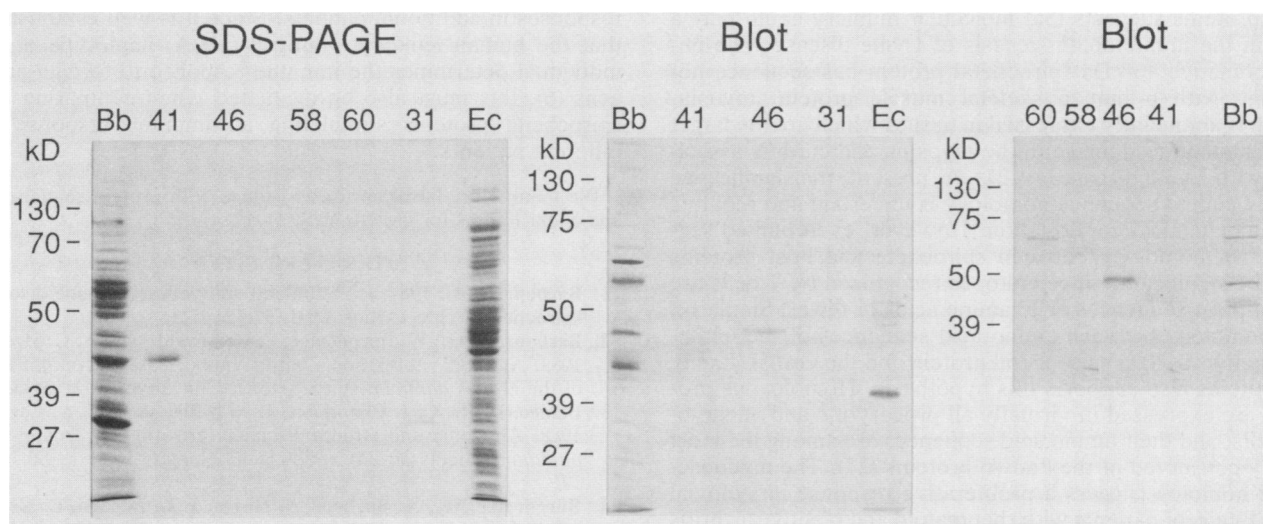


FIG. 2. Coomassie blue-stained SDS-10% PAGE of *B. burgdorferi* (Bb) and *E. coli* (Ec) lysate proteins and 31-, 41-, 46-, 58-, and 60-kDa proteins purified from *Borrelia burgdorferi*. The proteins were transferred to nitrocellulose, and immunoblotting was performed by using synovial fluid from a patient with late Lyme arthritis. The immunoblot of a smaller SDS-PAGE gel is also shown to demonstrate the reactivity of the four purified proteins with synovial fluid antibodies.

NH₂-TERMINAL AMINO ACID SEQUENCES

Bb41kD M I I N H N T S A I N A S K (N/E) (N/V) G I
 Bb46kD G F ? I Y E I K S V Q I
 Bb58kD A K D I Y F N E D A R K S L L G ? E
 Bb60kD A D A L G E K D I F (G/K) T N P L Y G G

HOMOLOGIES

Bb41kD ¹M I I N H N T S A I¹⁰
 Tp33kD ¹M I I N H N M S A M¹⁰

Bb46kD ¹G F ? I Y E I K S V Q I¹²
 ChkMy ⁴⁶⁸G F E I F E I N S F E Q L⁴⁸⁰
 HuMy ⁴⁶⁸G F E I F E Y N S L E Q L⁴⁷⁷

Bb58kD ¹A K D I Y F N E D A R K S L L G ? E¹⁸
 HuP1 ²⁷A K D Y K F G A D A R A I M L Q G V E⁴⁵
 EcGroEL ¹A K D V K F G N D A R V K M L R G V N²⁰
 Mb65kD ¹A K T I A Y D E E A R R G L E R G L N²⁰

FIG. 3. (Top) NH₂-terminal amino acid sequences of four immunoreactive *B. burgdorferi* (Bb) proteins. (Bottom) Sequence alignment of the 41-kDa *Borrelia* protein (Bb41kD) and the 33-kDa *Treponema pallidum* endoflagellar protein (Tp33kD; 3); the 46-kDa *Borrelia* protein (Bb46kD) and chicken smooth muscle (ChkMy; 26) and human skeletal muscle (HuMy; 8) myosin heavy chains; and the 58-kDa *Borrelia* protein (Bb58kD) with *E. coli* GroEL protein (EcGroEL; 11), human P1 (HuP1; 12), and the 65-kDa major antigen from *Mycobacterium bovis* BCG (Mb65kD; 22). Uncertain amino acid assignments are indicated by a question mark. Amino acid positions are indicated by numbers. Positions with identical or conservatively substituted amino acids are in boldface.

Alternatively, it is possible that the immune response to determinants on microbial organisms that are also found on host proteins may lead to chronic inflammatory responses deleterious to the host (18).

Our finding that the NH₂-terminal amino acid sequences of *B. burgdorferi* proteins inducing a humoral immune response are homologous with amino acid sequences present in human proteins suggests that molecular mimicry could play a role in the immunopathogenesis of Lyme disease. The immunoreactive 46-kDa spirochetal protein has sequence homology with a human skeletal muscle protein, myosin, which is abundantly expressed in tissues which are the target of inflammatory damage in the myositis and carditis associated with Lyme disease (19–21). At present, the significance of this limited sequence homology is uncertain and requires immunochemical confirmation. However, even limited segments of homology between spirochete and host proteins may be significant, since epitopes recognized by T cells are short linear sequences of 10 amino acids or fewer. Similarly, the homology between amino acid sequences of the immunoreactive 58-kDa spirochetal protein and the GroEL family of proteins also supports this hypothesis. Homologous proteins are expressed in virtually all procaryotic and eucaryotic cells, and their amino acid sequences are among the most highly conserved of the known proteins (27). The mycobacterial homolog triggers a proliferative response in synovial fluid T cells of patients with rheumatoid and reactive arthritis (9) and has an epitope, cross-reactive with a determinant on cartilage proteoglycan, that is recognized by a T-cell clone capable of causing an inflammatory arthritis in rats (24, 25). In addition, human T-cell lines reactive with the bacterial

GroEL protein will cross-react with the homologous human protein (14).

The shared determinant hypothesis can be fully evaluated only after epitopes on the immunoreactive *Borrelia* proteins recognized by B and T cells have been identified. Determination of which, if any, spirochetal protein is arthritogenic requires analysis of spirochetal proteins inducing immune responses in additional patients. Since it is well established that the human leukocyte antigen (HLA) haplotype of an individual determines the immune response to foreign antigens (6), this must also be evaluated when comparing the spirochetal proteins stimulating an immune response in different patients.

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