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Borrelia burgdorferi HSP70 Homolog: Characterization of an Immunoreactive Stress Protein

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The gene encoding an immunoreactive *Borrelia burgdorferi* HSP70 homolog was isolated and characterized. The predicted amino acid sequence of this spirochetal protein confirms that this gene encodes a member of the HSP70 family of proteins. Although there appears to be a single copy of this gene on the spirochetal chromosome, two distinct transcripts hybridizing to the *hsp70* probe are detected in RNA isolated from *B. burgdorferi*. The amount of spirochetal HSP70 RNA transcripts is shown to be thermally regulated. Antibodies in the serum of three Lyme arthritis patients and cloned T-cell lines isolated from one patient with Lyme arthritis recognize the expressed recombinant HSP70, indicating that it is an immunologically important spirochetal antigen. Antibodies in a rabbit antiserum, as well as antibodies in the serum of two of three Lyme arthritis patients examined, bound to expressed truncated recombinant HSP70s with 250 amino acids deleted from either the amino or carboxy terminus of the protein. However, antibodies in the serum of three Lyme arthritis patients, which were reactive with spirochetal HSP70, did not cross-react with human HSP70 proteins.

Stress (or heat shock) proteins of infecting bacterial or parasitic pathogens are major targets of the host humoral and cellular immune response (25, 33, 34). Because of the extensive sequence homology between heat shock proteins (HSPs) of the pathogen and the host (34), it is possible that the host immune response to the stress protein(s) of the pathogen will lead to an inflammatory response that cross-reacts with host tissues bearing the homologous protein(s). Evidence from experimental animal models of inflammatory diseases, including adjuvant arthritis (29) and insulitis in the NOD mouse (7), suggests that the immune response to a stress protein, HSP60, plays a role in the pathogenesis of chronic inflammation. T cells that recognize HSP60 homologs have been identified in the peripheral blood and synovial fluid of patients with rheumatoid (13), reactive (12), or Lyme (24) arthritis. However, there is no evidence at present indicating that the immune response to HSPs is involved in the pathogenesis of human inflammatory disease.

Although it has been far less extensively studied, immune reactivity to HSP70 homologs may also contribute to the pathogenesis of human inflammatory diseases. Human cells have at least five distinct members of the *hsp70* gene family, which are structurally and immunologically related but differ in their subcellular distribution and in whether their expression is constitutive or inducible (see reference 21 for a review). Members of this family include (i) a 75-kDa mitochondrial protein, (ii) a constitutively expressed 72-kDa protein, (iii) an inducible 72-kDa nuclear protein, (iv) an inducible 70-kDa protein, and (v) a constitutively expressed 74- to 78-kDa protein found in the endoplasmic reticulum and referred to as GRP78 or BiP. In human cells, HSP70 is induced and abundantly expressed in response to a variety of physiological or chemically induced stress conditions. The HSP70 proteins interact with other proteins in an ATP-dependent manner, and they have a role in regulating protein conformation and the assembly of oligomeric proteins and in protein translocation across membranes (see reference 21 for a review).

The immunodominant antigens recognized by antibodies in the serum of humans infected with a number of different protozoal pathogens, including *Plasmodium falciparum*, helminths, including *Schistosoma mansoni*, or bacteria, such as *Mycobacterium tuberculosis* and *M. leprae*, have been shown to be HSP70 homologs (see reference 32 for a review). However, the HSP70-reactive antibodies in other infectious diseases appear to recognize primarily epitopes that are not found on the homologous host protein (11). Nevertheless, patients with systemic lupus erythematosus have autoantibodies that recognize a constitutively expressed (73-kDa) member of the HSP70 family (20). In addition, active synthesis of HSP70 proteins occurs in osteoarthritic cartilage at physiologic temperatures (15).

We have been investigating the hypothesis that the host immune response, especially the T-cell response, to one or more spirochetal antigens plays an important role in the pathway leading to joint destruction in patients with chronic Lyme arthritis. Consistent with this possibility, we have previously shown that cloned T cells reactive with *B. burgdorferi* antigens, including spirochetal HSP60, can be isolated from the blood and synovial fluid of patients with chronic Lyme arthritis (24, 35, 36). *B. burgdorferi* has been shown by immunochemical criteria and by NH_2-terminal amino acid sequence analysis to have one or more proteins, whose synthesis is thermally regulated, that appear to be HSP70 homologs (5, 6, 17, 26). These proteins are bound by antibodies in the serum of patients with Lyme disease (18). To investigate the role of the immune response to spirochetal HSPs in the pathogenesis of Lyme disease, we have isolated and characterized the *B. burgdorferi* gene encoding an immunoreactive HSP70 homolog. We also demonstrate that

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serum antibodies and cloned human T-cell lines from patients with Lyme arthritis recognize the expressed recombinant HSP70 (rHSP70).

MATERIALS AND METHODS

Antibodies and antigens. The antiserum was prepared from a rabbit immunized with a sonicate of the B31 isolate of *B. burgdorferi* as previously described (18). Lysates of *Escherichia coli* expressing recombinant OspA and HSP70 proteins and lysates of the CA12 isolate of *B. burgdorferi* were prepared as described elsewhere (24). Purified *E. coli* DnaK and bovine brain HSP70 proteins were purchased from Epicentre Technologies, Madison, Wis., and StressGen Biotechnology Corp., Victoria, British Columbia, Canada, respectively. Purified *M. tuberculosis* HSP70 protein was obtained from R. Van der Zee. Human HSP70 proteins, purified from HeLa cells by affinity chromatography on ATP-agarose (30), were provided by William Welch, University of California, San Francisco. The HSP72 proteins were obtained from the soluble cytosolic fraction, and the fraction containing four human HSP70 homologs was purified from detergent-treated whole-cell extracts.

Patients. Patient 1 is a 14-year-old female resident of Suffolk County, N.Y., with a history of multiple tick bites, who developed an intermittent arthritis affecting her right knee. She had serum anti-*B. burgdorferi* antibodies in high titer as determined by enzyme-linked immunosorbent assay (ELISA), and immunoblotting indicated antibody binding to multiple spirochetal antigens. After 1 year of intermittent arthritic symptoms, the arthritis resolved after treatment with ceftriaxone for 2 weeks and subsequently with doxycycline for 2 months. The blood sample was obtained from patient 1 during a period of active arthritic inflammation, just prior to treatment with antibiotics. The clinical histories of Lyme arthritis patients 2 and 3, whose serum samples were used in immunoblotting, were described elsewhere (24). Four months after experiencing a tick bite, patient 2 developed a skin rash which spontaneously resolved after several weeks. Several months later he developed an asymmetric intermittent oligoarthritis, involving primarily the knees. The arthritis persisted for 2 years prior to referral to a rheumatologist. Evaluation by a rheumatologist in 1985 revealed a negative rheumatoid factor and assay for microagglutination to *Treponema pallidum*; synovial fluid aspirate had 7,500 leukocytes (75% polymorphonuclear leukocytes) with no bacteria or crystals. The diagnosis of Lyme arthritis was confirmed when serum antibody reactivity with *Borrelia* proteins in an ELISA (immunoglobulin G index, >2) was shown. Since treatment with a 10-day course of intravenous penicillin in March 1986, there has been total resolution of the arthritis. Blood from patient 2 was obtained 3 years after antibiotic treatment produced total resolution of his arthritic symptoms. Patient 3 was originally diagnosed as having Lyme arthritis at Yale University School of Medicine and was among the patients described in the initial studies on the clinical course of Lyme arthritis (27). An intermittent arthritis, involving multiple joints, and neuropathy were present for more than 10 years. Patient 3 had serum antibodies reactive with spirochetal antigens (ELISA titer of greater than 1/6,400 in November 1989). Treatment with intravenous ceftriaxone in November of 1989 produced near-complete resolution of the arthritic symptoms. Blood from patient 3 was obtained in April 1990, while the patient was experiencing a mild recurrence of polyarthralgia and fatigue. The control serum samples (CS1 and CS2) were obtained from healthy 25- and 27-year-old men without tick bite, arthritis, or symptoms of Lyme disease.

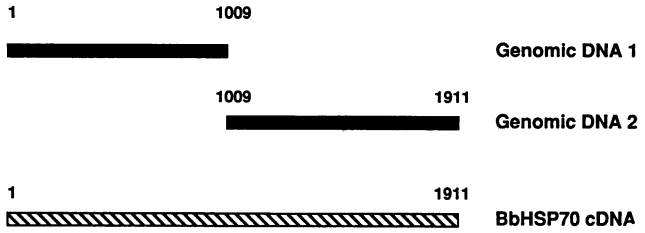

FIG. 1. Diagramatic representation of the two genomic fragments and full-length cDNA encoding the spirochetal HSP70 homolog.

Isolation of the genomic DNA encoding the *B. burgdorferi* HSP70 homolog. DNA prepared from the CA12 isolate of *B. burgdorferi* (24) was digested with the *Sau*3A restriction enzyme. Restriction fragments of greater than 2 kb were purified from an agarose gel and ligated into *Bam*HI-digested Bluescript KS+ plasmid (pBSKS$^+$; Stratagene, La Jolla, Calif.). *E. coli* JM109 was transformed by the ligation reaction to prepare the genomic library (3). A single-stranded form of the library was prepared by using helper phage R408 as specified by the manufacturer (Stratagene) as a template for polymerase chain reaction amplification. Spirochetal *hsp70* DNA probes were generated from the single-stranded *B. burgdorferi* genomic library by polymerase chain reaction amplification with a pair of oligonucleotide primers. One oligonucleotide primer (5′-GGT ATT ACC AAC TCT GGT GTA GCG ATT ATG-3′) corresponded to the NH_2-terminal amino acid sequence of the 71-kDa spirochetal protein (17). The choice of nucleotides at the third position of a codon and for amino acids not identified during sequencing of the protein was biased according to the nucleotide sequence of the aligned region of the *E. coli dnaK* gene (4). The antisense oligonucleotide was the T7 oligonucleotide primer (Stratagene). Amplifications were performed by using a thermal cycler and reagents as specified by the manufacturer (Perkin-Elmer/Cetus, Norwalk, Conn.) with the following cycle parameters: 1 min at 94°C for denaturation, 2 min at 37°C for annealing, and 3 min at 42°C for extension. The temperatures for annealing and extension were reduced because of the low percentage of guanine and cytosine nucleotides in the *B. burgdorferi* genome. The 1,200- and 700-bp amplified DNA segments were purified from agarose gels, digested with *Sac*I, and ligated into *Sac*I- and *Sma*I-digested pBSKS$^+$ plasmid by procedures described elsewhere (24). The two amplified DNA segments had identical 5′ nucleotide sequences, which were homologous to the *E. coli* DnaK (4) protein. The 1,200-bp fragment was radiolabeled with [α-^{32}P]dCTP by random priming (Boehringer, Mannheim, Germany) for use as a probe to isolate genomic DNA fragments from the *B. burgdorferi* library by colony hybridization. After transfer of bacterial colonies to Hybond-N (Amersham, Arlington Heights, Ill.) membranes, alkaline denaturation, and cross-linking with UV light as specified by the manufacturers, hybridizations were performed by overnight incubation of the membranes at 65°C in a solution containing 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), 0.1% sodium PP_i, 100 μg of denatured salmon sperm DNA per ml, and ^{32}P-labeled *hsp70* probe. The membranes were then washed at 65°C in a solution containing 0.2× SSC, 0.2% SDS, and 0.1% sodium

PP_i. Two hybridizing plasmids with *Borrelia* genomic DNA inserts of greater than 2,000 bp were isolated. One 2,100-bp genomic fragment contained nucleotides 1 to 1009 of the coding region of spirochetal *hsp70*, and a second, 2,200-bp genomic fragment contained nucleotides 1009 to 1911 (Fig. 1).

Preparation of expressed recombinant *B. burgdorferi* HSP70s. The following oligonucleotides, synthesized on a model 391 oligonucleotide synthesizer (Applied Biosystems, Foster City, Calif.), were used in the construction of the expressed recombinant HSP70 (5′ → 3′):

BbHSP70-19: GGC CCC GGG ATG GGC AAA ATA ATA GGT ATT
Met-1

BbHSP70-24: GGC CCC GGG TCA CTA TTT TTT ATC CTC
* * Lys

*Sma*I restriction sites (underlined) were placed at the 5′ and 3′ ends, respectively, of the amplified DNA to facilitate ligation of the amplified DNA into a plasmid vector. BbHSP70-24 is an antisense oligonucleotide with stop codons (*) after Lys-635. BbHSP70-19 is a sense oligonucleotide with an ATG initiation codon at position 1.

Total RNA, isolated from a culture of the CA12 isolate (28), was reverse transcribed (36) by using BbHSP70-24 as the oligonucleotide primer. A full-length copy of the HSP70 open reading frame was amplified from the resulting cDNA by using the above two oligonucleotide primers. The 1,900-bp amplified DNA was purified from an agarose gel, digested with *Sma*I, and ligated into *Sma*I-digested prokaryotic expression plasmid pKK223-3 (Pharmacia, Piscataway, N.J.) as described elsewhere (24). The resulting plasmid encoding spirochetal HSP70 was referred to as pHSP70-A.

Truncated forms of spirochetal HSP70, as well as the full-length HSP70, were expressed in a different prokaryotic expression plasmid and bacterial host. The following oligonucleotides were used in the construction of these rHSP70s:

HSPJA-27: GGC ATC CAT ATG GGC AAA ATA ATA GGT ATT
Met-1

HSPJA-28: GGC ATC GGA TCC TCA CTA TTT TTT ATC CTC
* *

HSPJA-29: GGC ATC GGA TCC TTA TTA TGA TGA TGA TTC AAT TCT AAT
* *

HSPJA-30: GGC ATC GGA TCC TTA TTA AGC TCC AAT TGC TAC AGC TTC
* *

HSPJA-31: GGC ATC CAT ATG GCT CTT CAA AGA CTC AAA
Met-252

*Nde*I and *Bam*HI restriction sites (underlined) were placed at the 5′ and 3′ ends, respectively, of the oligonucleotides to facilitate ligation of the amplified DNA into the plasmid vector. HSPJA-28, HSPJA-29, and HSPJA-30 are antisense oligonucleotides with stop codons (*) after Lys-635, Ser-500, and Ala-370. HSPJA-27 and HSPJA-31 are sense oligonucleotides with ATG initiation codons at positions 1 and 252, respectively. The full-length and truncated *hsp70* genes were amplified by using plasmid pHSP70-A as template DNA (Table 1).

Polymerase chain reaction amplification and purification of the amplified DNAs were performed as described above. The purified DNAs were ligated into the prokaryotic expression plasmid pET3a (Novagen, Madison, Wis.), digested with *Nde*I and *Bam*HI restriction enzymes, prior to transformation of *E. coli* BL21(DE3)pLYS S. Bacterial cultures transformed with control plasmid pET3a or the HSP70-encoding plasmids were induced with 3 mM isopropyl-β-D-thiogalactopyranoside (USB, Cleveland, Ohio), and lysates of the harvested cells were prepared as previously described (24).

TABLE 1. Oligonucleotide primers used in *hsp70* gene amplification

Plasmid	Oligonucleotides	HSP70 amino acids encoded
pHSP70-1	HSPJA-27 + HSPJA-28	Met-1 to Lys-635
pHSP70-2	HSPJA-27 + HSPJA-29	Met-1 to Ser-500
pHSP70-3	HSPJA-27 + HSPJA-30	Met-1 to Ala-370
pHSP70-4	HSPJA-31 + HSPJA-28	Met-252 to Lys-635

DNA sequence analysis. The nucleotide sequences of both strands of the coding region of the two genomic DNAs and regions of two independently isolated cDNAs encoding the HSP70 open reading frame were determined by using the dideoxy method with modified T7 DNA polymerase (Stratagene). DNA sequencing reactions were performed with synthetic oligonucleotides as primers. The sequence spanning the junction of the two genomic fragments was confirmed by sequencing this region of two independently obtained cDNAs encoding the HSP70 open reading frame. Translation of the *hsp70* nucleotide sequence and alignment of the amino acid sequences were performed by using PC/GENE computer software (IntelliGenetics, Mountain View, Calif.).

Culture conditions for analysis of spirochetal heat stress response. Cultures of CA12 and B31 strains of *B. burgdorferi* were grown in BSKII medium (1) at 28°C to a concentration of 5×10^7 to 1×10^8 cells per ml, harvested by centrifugation (3,000 × *g*), and immediately resuspended in fresh medium at a concentration of 1.5×10^8 cells per ml. The cultures were aliquoted and incubated at either 28 or 38°C for 4 h before being harvested for RNA preparation.

RNA and DNA blot analysis. RNA was prepared as described previously (28). RNA species were transferred from formaldehyde-containing agarose gels to nylon membrane (Nytran, 0.45 μm; Schleicher & Schuell, Keene, N.H.) as described previously (19). Each lane contained 10 μg of RNA, quantitated by measurement of A_{260}. DNA was purified from the B31 isolate of *B. burgdorferi* by previously described procedures (3). DNA blotting was performed by the method of Maniatis et al. (19). Plasmids containing nucleotides 1 to 1009 of spirochetal *hsp70* or nucleotides 1 to 1638 of spirochetal *hsp70* (24) were radiolabeled with [α-^{32}P]dATP by random priming for use as hybridization probes (8).

Pulsed-field gel electrophoresis. Agarose blocks of four isolates of *B. burgdorferi* (B31, 297, IP-2, and Arc) were prepared as described by Ferdows and Barbour (9) with minor modifications. Spirochetes were grown to a cell density of 10^8 cells per ml. Cells were harvested by centrifugation at 8,000 × *g* for 15 min at 20°C and then resuspended in TN buffer (50 mM Tris, 150 mM NaCl [pH 8.0]) at 37°C. An equal volume of molten 1% agarose (SeaKem Incert; FMC Corp.) in TN buffer was added to the cell suspension, and the mixture was poured into acrylic casting wells. After the blocks solidified, they were first immersed in the lysis solution, which in its complete form consisted of 50 mM Tris, 50 mm EDTA, and 1% SDS (pH 8.0) with 1 mg of proteinase K (Boehringer) per ml, and then incubated at 50°C for 16 to 24 h. The DNA concentration in the melted blocks was determined by microfluorimetry (Hoefer) with calf thymus DNA as a standard. Agarose blocks were stored

A

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                                                                    60
ATGGGCAAAATAATAGGTATTGACCTAGGAACAACAAACTCATGCGTAGCTATAATGGAG
 M  G  K  I  I  G  I  D  L  G  T  T  N  S  C  V  A  I  M  E
                                                                   120
CATGGAAAACCAGTTGTAATACAAAATTCAGAAGGGGGAAGAACTACTCCATCTATTGTT
 H  G  K  P  V  V  I  Q  N  S  E  G  G  R  T  T  P  S  I  V
                                                                   180
GCTTACACAAATAAAGGCGAAAGACTTGTGGGACAAGTCGCAAAAAACCAAATGGTTACA
 A  Y  T  N  K  G  E  R  L  V  G  Q  V  A  K  N  Q  M  V  T
                                                                   240
AACCCTGAAAACACAATTTATTCTATTAAAAGATTTATGGGAAGAAGATTTGAAGAGGTT
 N  P  E  N  T  I  Y  S  I  K  R  F  M  G  R  R  F  E  E  V
                                                                   300
GCGAGCGAAATAAAAATGGTTCCTTATAAAATAGAAAAGGGTCTAAATGGCGACGCACGA
 A  S  E  I  K  M  V  P  Y  K  I  E  K  G  L  N  G  D  A  R
                                                                   360
GTAAACATTTCCAATATAAAAAAGCAAATGTCACCGCCTGAGATCTCAGCAGCAACTCTT
 V  N  I  S  N  I  K  K  Q  M  S  P  P  E  I  S  A  A  T  L
                                                                   420
ACAAAAATGAAAGAAACAGCAGAGGCTTACTTAGGTGAAAAAGTTACAGAGGCTGTCATT
 T  K  M  K  E  T  A  E  A  Y  L  G  E  K  V  T  E  A  V  I
                                                                   480
ACAGTTCCTGCTTACTTCAATGATGCTCAAAGACAAGCCACAAAAGATGCTGGCAAAATA
 T  V  P  A  Y  F  N  D  A  Q  R  Q  A  T  K  D  A  G  K  I
                                                                   540
GCAGGTCTTGAAGTAAAAAGAATTGTTAACGAACCAACTGCTGCTGCTCTTGCTTATGGA
 A  G  L  E  V  K  R  I  V  N  E  P  T  A  A  A  L  A  Y  G
                                                                   600
ATTGAAAAAAAAACACGAAGAGATTGTTGCTGTTTACGATCTTGGTGGAGGAACATTTGAC
 I  E  K  K  H  E  E  I  V  A  V  Y  D  L  G  G  G  T  F  D
                                                                   660
ATTTCAATATTGGAACTTGGAGATGGTGTTTTTGAAGTAAAGTCAACCAACGGAGACACA
 I  S  I  L  E  L  G  D  G  V  F  E  V  K  S  T  N  G  D  T
                                                                   720
CATCTTGGTGGTGACAATTTTGACGACGAAATAATAAAACATTTAATTTCAGAATTCAAA
 H  L  G  G  D  N  F  D  D  E  I  I  K  H  L  I  S  E  F  K
                                                                   780
AAAGAAAGCGCTATTGATTTATCAAACGACAAAATGGCTCTTCAAAGACTCAAAGAAGCA
 K  E  S  A  I  D  L  S  N  D  K  M  A  L  Q  R  L  K  E  A
                                                                   840
GCTGAAAAAGCTAAAATAGAACTCTCAGGCGCTCAAGAAGCTTCAATAAATCTTCCATTT
 A  E  K  A  K  I  E  L  S  G  A  Q  E  A  S  I  N  L  P  F
                                                                   900
ATTACAGCAGATGCAAATGGTCCAAAACACTTACAATATACTCTAACAAGAGCAAAATTC
 I  T  A  D  A  N  G  P  K  H  L  Q  Y  T  L  T  R  A  K  F
                                                                   960
GAACAAATGGTAGACCACTTAGTTCAAAAAACAAAAGAACCATGCCTTAAAGCTATTAAA
 E  Q  M  V  D  H  L  V  Q  K  T  K  E  P  C  L  K  A  I  K
                                                                  1020
GATGCTGGGCTTAAAGCTTCTGATATTAATGAAGTAATACTTGTAGGTGGATCAACAAGA
 D  A  G  L  K  A  S  D  I  N  E  V  I  L  V  G  G  S  T  R
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                                                                  1080
ATTCCTGCTATTCAAAAAATTGTAAAAGATATATTTGGACAAGATCCTAACAAAGGAGTA
 I  P  A  I  Q  K  I  V  K  D  I  F  G  Q  D  P  N  K  G  V
                                                                  1140
AATCCAGATGAAGCTGTAGCAATTGGAGCTGCTATTCAGGGTGGCATTCTAACAGGAGAA
 N  P  D  E  A  V  A  I  G  A  A  I  Q  G  G  I  L  T  G  E
                                                                  1200
ACTAAAGACATGGTACTCCTTGACGTTACTCCACTCTCACTAGGAATAGAAACACTAGGC
 T  K  D  M  V  L  L  D  V  T  P  L  S  L  G  I  E  T  L  G
                                                                  1260
GGGGTTATGACTAAACTTATTGAACGAAACACCACAATTCCTACAAAAAAAAGTCAAGTA
 G  V  M  T  K  L  I  E  R  N  T  T  I  P  T  K  K  S  Q  V
                                                                  1320
TTCTCCACAGCTGCTGACAATCAAACCTCTGTTGATATTAAAGTCCTTCAAGGTGAACGT
 F  S  T  A  A  D  N  Q  T  S  V  D  I  K  V  L  Q  G  E  R
                                                                  1380
GAAATGGCAGCACAAAACAGAATACTTGGTAATTTTATACTTGATGGAATACCAGCAGCA
 E  M  A  A  Q  N  R  I  L  G  N  F  I  L  D  G  I  P  A  A
                                                                  1440
CCAAGAGGAGTGCCTCAAATTGAAGTTAGCTTTGACATTGATGCTAATGGAATAGTTCAT
 P  R  G  V  P  Q  I  E  V  S  F  D  I  D  A  N  G  I  V  H
                                                                  1500
GTGTCTGCAAAAGATATGGGAACAGGCAAAGAACAAAAAATTAGAATTGAATCATCATCA
 V  S  A  K  D  M  G  T  G  K  E  Q  K  I  R  I  E  S  S  S
                                                                  1560
GGACTCTCTGAATCAGAAATAGATCGAATGGTAAAAGATGCAGAAGCTCATGCGGAAGAA
 G  L  S  E  S  E  I  D  R  M  V  K  D  A  E  A  H  A  E  E
                                                                  1620
GATAAAAAATTAAAAGAAAATATTGAAGCAAAAAATACAGCTAATTCTTTAATTTATCAA
 D  K  K  L  K  E  N  I  E  A  K  N  T  A  N  S  L  I  Y  Q
                                                                  1680
ACAGAAAAATCATTAAAAGAATATTCTGAAAAAATTTCAAGCGAAGACAAAGAAGCTATT
 T  E  K  S  L  K  E  Y  S  E  K  I  S  S  E  D  K  E  A  I
                                                                  1740
GAAAGTAAAATAAAAGAATTAAAAGAAAGTCTTGAAAAAGAAGACATTTCACTTATAAAA
 E  S  K  I  K  E  L  K  E  S  L  E  K  E  D  I  S  L  I  K
                                                                  1800
TCTAGAACAGAAGAACTTCAAAAAGCTTCTTACAAAATAGCTGAGATGATGTATAAAGAT
 S  R  T  E  E  L  Q  K  A  S  Y  K  I  A  E  M  M  Y  K  D
                                                                  1860
TCTTCCCAGCAAAATGCAAACAGCCAACAAGAAAATGGCCCACAAAGCAATACAAGCGAA
 S  S  Q  Q  N  A  N  S  Q  Q  E  N  G  P  Q  S  N  T  S  E
                                                                  1911
GAGGGTAAAGAGGCTGATTATGAGGTTGTTGACGAGGATAAAAAATAGTGA
 E  G  K  E  A  D  Y  E  V  V  D  E  D  K  K  -  -
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B

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BBHSP70  MGKI--IGIDLGTTNSCVAIMEHGKPVVIQNSEGGRTTPSIVAYTNKGER   48
ECDNAK   MGKI--IGIDLGTTNSCVAIMDGTTPRVLENAEGDRTTPSIIAYTQDGET   48
HSHSP70  MAKAAAVGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFTDT-ER   49
         *.*  .******* ***.... ..  ...*..*.***** .*.*.. *

BBHSP70  LVGQVAKNQMVTNPENTIYSIKRFMGRRF--EEVASEIKMVPYKIEK-GL   95
ECDNAK   LVGQPAKRQAVTNPQNTLFAIKRLIGRRFQDEEVQRDVSIMPFKIIA-AD   97
HSHSP70  LIGDAAKNQVALNPQNTVFDAKRLIGRKFGDPVVQSDMKHWPFQVINDGD   99
         *.*. **.* . **.**... **..**.*    *.....  *...   .

BBHSP70  NGDARVNISNIKKQMSPPEISAATLTKMKETAEAYLGEKVTEAVITVPAY  145
ECDNAK   NGDAWVEVKGQK--MAPPQISAEVLKKMKKTAEDYLGEPVTEAVITVPAY  145
HSHSP70  KPKVQVSYKGETKAFYPEEISSMVLTKMKEIAEAYLGYPVTNAVITVPAY  149
         . .. *. .. .  . * .**. .*.***..**.***  **.********

BBHSP70  FNDAQRQATKDAGKIAGLEVKRIVNEPTAAALAYGIEK-KHEE-IVAVYD  193
ECDNAK   FNDAQRQATKDAGRIAGLEVKRIINEPTAAALAYGLDK-GTGNRTIAVYD  194
HSHSP70  FNDSQRQATKDAGVIAGLNVLRIINEPTAAAIAYGLDRTGKGERNVLIFD  199
         ***.********* ****.* **.*******.***...   ..  . ..*

BBHSP70  LGGGTFDISILEL----GDGVFEVKSTNGDTHLGGDNFDDEIIKHLISEF  239
ECDNAK   LGGGTFDISIIEIDEVDGEKTFEVLATNGDTHLGGEDFDSRLINYLVEEF  244
HSHSP70  LGGGTFDVSILTID----DGIFEVKATAGDTHLGGEDFDNRLVNHFVEEF  245
         *******.**...     . .*** .*.*******..**. .......**

BBHSP70  KKESAIDLSNDKMALQRLKEAAEK-AKIELSGAQEASINLPFITADANGP  288
ECDNAK   KKDQGIDLRNDPLAMQRLKEAAEK-AKIELSSAQQTDVNLPYITADATGP  293
HSHSP70  KRKHKKDISQNKRAVRRLRTACERPEGIDFYTSITRARFEELAKRTLSSS  295
         *..   *....  *..**..* *. . *.. ..   .     . . ...

BBHSP70  KHLQYTLTRAKFEQMVDHLVQKTKEPCLKAIKDAGLKASDINEVILVGGS  338
ECDNAK   KHMNIKVTRAKLESLVEDLVNRSIEPLKVALQDAGLSVSDIDDVILVGGQ  343
HSHSP70  TQASLEI-----DSLCSDLFRSTLEPVEKALRDAKLDKAQIHDLVLVGGS  340
         ..   ..     . . ..* ... **   *..** *. ..*....****

BBHSP70  TRIPAIQKIVKDIF-GQDPNKGVNPDEAVAIGAAIQGGILTGE----TKD  383
ECDNAK   TRMPMVQKKVAEFF-GKEPRKDVNPDEAVAIGAAVQGGVLTGD----VKD  388
HSHSP70  TRIPKVQKLLQDFFNGRDLNKSINPDEAVGYGAAVQAAILMGDKSENVQD  390
         **.* .** . ..* * . .*..******. ***.*...* *.    ..*

BBHSP70  MVLLDVTPLSLGIETLGGVMTKLIERNTTIPTKKSQVFSTAADNQTSVDI  433
ECDNAK   VLLLDVTPLSLGIETMGGVMTTLIAKNTTIPTKHSQVFSTAEDNQSAVTI  438
HSHSP70  LLLLDVAPLSLGLETAGGVMTALIKRNSTIPTKQTQIFTTYSDNQPGVLI  440
         ..****.******.** ***** ** .*.******..*.*.* .***..* *

BBHSP70  KVLQGEREMAAQNRILGNFILDGIPAAPRGVPQIEVSFDIDANGIVHVSA  483
ECDNAK   HVLQGERKRAADNKSLGQFNLDGINPAPRGMPQIEVTFDIDADGILHVSA  488
HSHSP70  QVYEGERAMTKDNNLLGRFELSGIPPAP-GVPQIEVTFDIDANGILNVTA  489
         .* .*** .. .*. **.* *.** .** *.******.*****.**..*.*

BBHSP70  KDMGTGKEQKIRIESSSG-LSESEIDRMVKDAEAHAEEDKKLKENIEAKN  532
ECDNAK   KDKNSGKEQKITIKASSG-LNEDEIQKMVRDAEANAEADRKFEELVQTRN  537
HSHSP70  TDKSTGKANKITITNDKGRLSKEEIERMVQEAEKYKAEDEVQRERVSAKN  539
         .*...**..** *....* *...**..**..**   ..*    * . ..*

BBHSP70  TANSLIYQTEKSLKEYS--EKISSEDKEAIESKIKELKESLEKE---DIS  577
ECDNAK   QGDHLLHSTRKQVEEAG--DKLPADDKTAIESALTALETALKGE---DKA  582
HSHSP70  ALESYAFNMKSAVEDEGLKGKISEADKKKVLDKCQEVISWLDANTLAEKD  589
           .      . ... .  .*....**. . .   .. * .  .  .

BBHSP70  LIKSRTEELQKASYKIAEMMYKDSSQQNANSQQENGPQSNTSEEGKEADY  627
ECDNAK   AIEAKMQELAQVSQKLMEIAQQQHAQQQTAGA-DASANNAKDDDVVDAEF  631
HSHSP70  EFEHKRKELEQVCNPIISGLYQGAG---GPGPGGFGAQGPKGGSGSGPTI  636
         .. . .**....  . .   .  .  . .. . .... ....  ...

BBHSP70  EVVDEDKK   635
ECDNAK   EEVK-DKK   638
HSHSP70  EEVD----   640
         * *.
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FIG. 2. (A) Nucleotide sequence for and predicted amino acid sequence of the *B. burgdorferi* (CA12 isolate) HSP70 homolog. (B) Alignment of the predicted amino acid sequences of the *B. burgdorferi* (BBHSP70), *E. coli* (ECDNAK), and human (HSHSP70) HSP70 homologs. Symbols: *, positions where all three proteins have the same amino acid; ·, positions with conserved amino acids; –, gaps introduced into the sequence.

in 10 mM Tris–1 mM EDTA (pH 8.0) (TE buffer) at 4°C until use.

Electrophoresis was performed in a Bio-Rad CHEF-DRII system. The DNA blocks were washed in TE buffer and then loaded into wells of a 1% agarose gel with 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA) for electrophoresis with a pulse time of 60 s at 200 V for 18 h. Yeast chromosome (*Saccharomyces cerevisiae* YNN 295) from Bio-Rad was used as molecular weight markers.

Immunoblotting. One-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) separation of proteins was performed by using 10% acrylamide gels under reducing conditions as previously described (24). Lysates of *B. burgdorferi* or *E. coli* (5 to 20 μg) and purified proteins (1 to 2 μg) were used in each lane. Protein transfer to nitrocellulose, the blocking procedure, incubation with a 1:100 dilution of preabsorbed serum, and chemiluminescent detection of bound antibody by using horseradish peroxidase-conjugated $F(ab')_2$ anti-human immunoglobulin G (Amersham) were performed as specified by manufacturer.

Preparation of cloned T cells and lymphoblastoid B-cell lines. Cloned T-cell lines reactive with *B. burgdorferi* antigens were isolated from peripheral blood mononuclear cells obtained from patient 1 exactly as previously described (24). The cultures were cloned by limiting dilution by using the previously described feeder mixture without *Borrelia* antigen. Autologous lymphoblastoid B-cell lines were also prepared from peripheral blood mononuclear cells of this patient by in vitro infection with Epstein-Barr virus (24). All cloned T-cell lines and lymphoblastoid B-cell lines were

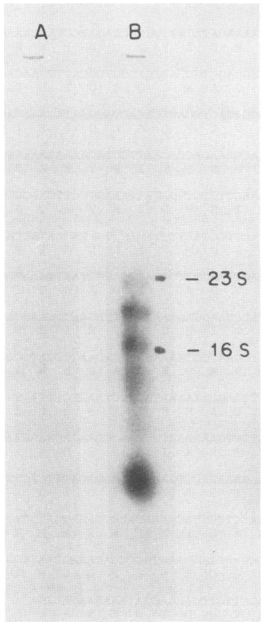

FIG. 3. RNA blot analysis of *hsp70* transcripts. RNA (10 μg) isolated from *E. coli* (A) or the B31 isolate of *B. burgdorferi* (B) was hybridized to ^{32}P-labeled *hsp70* probe. The positions of 23S and 16S rRNAs are indicated.

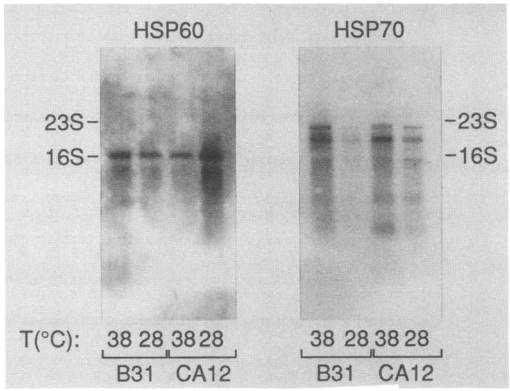

FIG. 4. Thermal induction of HSP70 RNA transcripts. RNA (10 μg) prepared from the B31 or CA12 isolate of *B. burgdorferi* cultured at 28 or 38°C was hybridized to ^{32}P-labeled *hsp60* or *hsp70* probe. The positions of 23S and 16S rRNAs are indicated.

cultured in Yssel's medium (24) supplemented with 1% AB$^+$ human serum (Irvine Scientific, Santa Ana, Calif.).

Analysis of the cloned T cells and lymphoblastoid B-cell lines. Cellular proliferation was measured by using a 72-h [^{3}H]thymidine assay as described previously (35). Analysis of cells for the expression of cell surface antigens was performed by flow cytometry with a FACScan (Becton-Dickinson, Mountain View, Calif.) as previously described (35). The following monoclonal antibodies against the antigens indicated in parentheses were used: 6G4 (CD2), RIV-6 (CD4), SPV-T3b (CD3), SPV-T8 (CD8), BF1 (TCRαβ; T Cell Sciences, Cambridge, Mass.), and TCRδ1 (TCR δ chain).

RESULTS

Isolation and DNA sequence analysis of the *B. burgdorferi* gene encoding the HSP70 homolog. The nucleotide sequence of the gene encoding the *B. burgdorferi* HSP70 homolog and the predicted amino acid sequence are presented in Fig. 2A. A 1,905-bp open reading frame commences with a methionine codon and encodes a 635-amino-acid protein with a predicted molecular weight of 69,260. Two sequential stop codons at nucleotides 1906 to 1911 terminate the open reading frame. The amino acids at positions 2 to 20 are identical to those at the NH_2 terminus of the purified 72-kDa spirochetal protein (17). Alignment of the predicted amino acid sequence of this spirochetal HSP70 with the *E. coli* DnaK (4) and human HSP70 (14) protein sequences indicates that the spirochetal protein is an HSP70 homolog (Fig. 2B). This amino acid alignment indicates that spirochetal HSP70 has 58.7 and 45.8% identity with the *E. coli* DnaK (4) and human HSP70 (14) proteins, respectively. The amino acid sequences of the eukaryotic and spirochetal HSP70 homologs are most divergent at their carboxy termini.

RNA and genomic DNA blot analysis. RNA blot analysis with the spirochetal *hsp70* probe revealed hybridization to two RNA transcripts of approximately 2.0 and 2.5 kb, present in RNA isolated from *B. burgdorferi* (Fig. 3). Hybridizations performed with ^{32}P-labeled probes containing nucleotides 1 to 1009, 1009 to 1911, or 1881 to 1911 of spirochetal *hsp70* yielded identical results (data not shown). The *hsp70* probe did not hybridize to RNA isolated from *E. coli* (Fig. 3). The presence of two RNA transcripts hybridizing to the *hsp70* probe is not due to degradation of the spirochetal RNA, since only one RNA species hybridizes to the spirochetal *hsp60* probe (Fig. 4). RNA blot analysis also reveals that the amount of HSP70 RNA is thermally regulated. In two different isolates of *B. burgdorferi*, the amount of HSP70 RNA increases when the temperature of spirochete cultivation is increased from 28 to 38°C (Fig. 4). It does appear that there is constitutively more HSP70 RNA in the lower-passage CA12 isolate than in the higher-passage B31 isolate. In contrast, there is no change in the amount of spirochetal HSP60 RNA in response to an elevation in temperature (Fig. 4).

The *B. burgdorferi* genome contains a 950-kb linear duplex chromosome (9), as well as linear and circular plasmids (2), which can be separated by using pulsed-field gel electrophoresis. To determine whether the spirochetal *hsp70* gene was located on the chromosome or on a plasmid, *B. burgdorferi* DNA was separated by pulsed-field gel electrophoresis and hybridized to an *hsp70* probe. This probe hybridized to chromosomal DNA and not to linear and circular plasmid DNA of four different isolates of *B. burgdorferi* (Fig. 5). Therefore, HSP70 is chromosomally encoded. The 245-kb band hybridizing to the *hsp70* probe in Fig. 5, lane 4, is likely to be sheared chromosomal DNA. As previously shown by Ferdows and Barbour (9), the ethidium bromide-stained DNA migrating between 100 and 945 kb in this figure is supercoiled spirochetal plasmid DNA.

The pattern of hybridization of the *hsp70* probe to spirochetal genomic DNA digested with *Eco*RI, *Bam*HI, or *Hin*dIII is shown in Fig. 6. This pattern of hybridization indicates that there is a single copy of the *hsp70* gene on the spirochetal chromosome. The nucleotide sequence (Fig. 2) indicates that there are two *Eco*RI sites (at nucleotide positions 712 and 1019), three *Hin*dIII sites (at nucleotide positions 818, 974, and 1763), and no *Bam*HI sites within the *hsp70* gene. Thus, *Eco*RI digestion of spirochetal DNA generates three *hsp70* restriction fragments, and the *hsp70* probe containing nucleotides 1 to 1009 could hybridize to

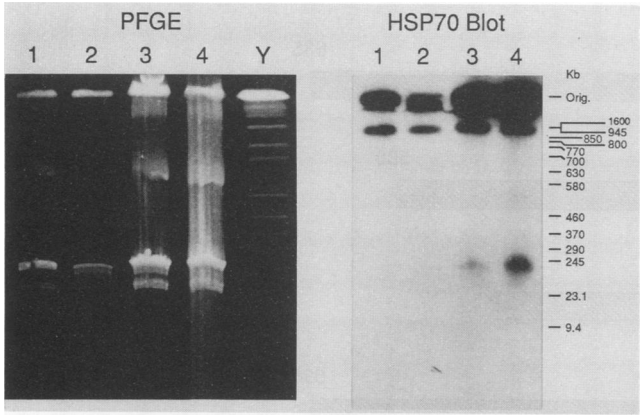

FIG. 5. The *hsp70* gene is on the *B. burgdorferi* chromosome. DNA prepared from four isolates of *B. burgdorferi*, B31 (lane 1), 297 (lane 2), IP-2 (lane 3), and Arc (lane 4), and yeast chromosomal markers (Y) were separated by pulsed-field gel electrophoresis, and visualized on an ethidium bromide-stained agarose gel. The gel was then blotted and hybridized to ^{32}P-labeled *hsp70* probe. The positions of molecular size markers are indicated to the right of the blot.

two of the fragments. This *hsp70* probe hybridized to only one *Eco*RI spirochetal DNA fragment, probably because the smaller *Eco*RI fragment (307 bp) was not retained on the gel. Similarly, the *hsp70* probe hybridized to a single *Hin*dIII restriction fragment, also resulting from lack of retention of the smaller (156-bp) *Hin*dIII fragment on the gel. Consistent with the lack of *Bam*HI restriction sites in the *hsp70* gene, the *hsp70* probe hybridized to only one *Bam*HI restriction fragment.

Immunoreactive expressed recombinant full-length and truncated spirochetal HSP70s. A prokaryotic expression plasmid (pHSP70-A) containing the spirochetal *hsp70* gene was prepared. SDS-PAGE analysis of a lysate of *E. coli* transfected with this plasmid demonstrates the presence of an expressed recombinant 70-kDa protein (rHSP70) (Fig. 7). This protein, which is not seen in lysates of *E. coli* transfected with a control plasmid, is bound by antibodies in the serum of a rabbit immunized with *B. burgdorferi*. Serum samples from three patients with chronic Lyme arthritis, with a high titer of anti-*Borrelia* antibodies as determined by an ELISA, were tested for binding to the expressed recombinant HSP70 protein by immunoblotting. All of these serum samples had antibodies reactive with the rHSP70 protein (Fig. 7). Antibody-reactive proteins of molecular mass less than 70 kDa in the rHSP70 lysate are likely to result from degradation of the expressed recombinant spirochetal protein since they are not found in the control lysate. In contrast, serum samples obtained from two healthy controls, without current or previous symptoms consistent with Lyme disease, did not react with the expressed rHSP70 or with *B. burgdorferi* lysate proteins (representative data in Fig. 7).

Recombinant plasmids encoding truncated *B. burgdorferi* HSP70 were prepared and expressed in *E. coli* for use in epitope localization. pHSP70-2 through pHSP70-4 are prokaryotic expression plasmids encoding the indicated portions of spirochetal HSP70 (Fig. 8A). Expressed proteins, of relative mobility consistent with the predicted molecular mass of the truncated HSP70 they encode, are found in lysates of *E. coli* transfected with pHSP70-2, pHSP70-3, and pHSP70-4 (Fig. 8B). The expressed recombinant proteins are bound by antibodies in the rabbit antiserum (Fig. 8B).

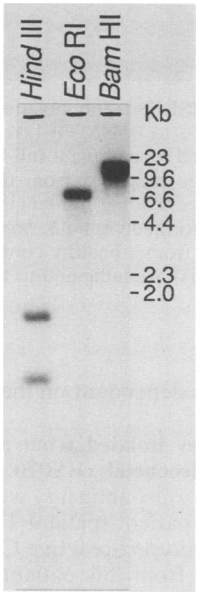

FIG. 6. Genomic DNA blot analysis. Aliquots of the B31 isolate of *B. burgdorferi* DNA were digested with *Eco*RI (3 μg), *Bam*HI (3 μg) or *Hin*dIII (10 μg), electrophoresed in 1% agarose, blotted, and hybridized with a ^{32}P-labeled *hsp70* probe containing nucleotides 1 to 1911. Sizes of DNA standards are shown to the left.

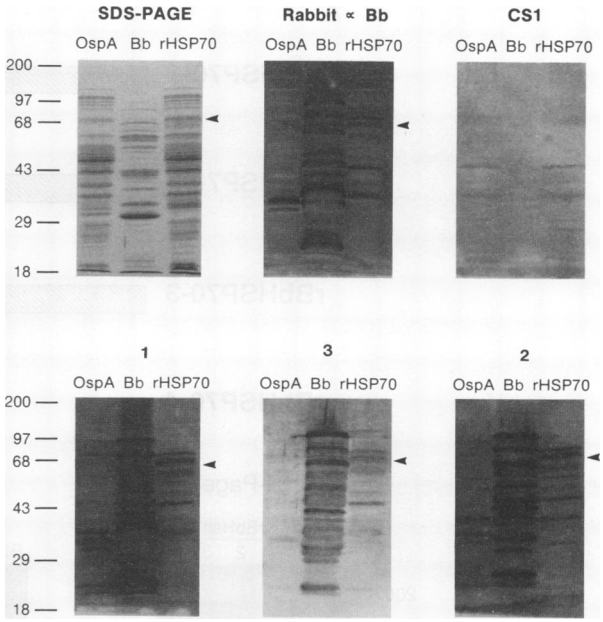

FIG. 7. Immunoblot analysis of antibody reactivity with an expressed recombinant *B. burgdorferi* HSP70. Coomassie blue-stained SDS-polyacrylamide gels (10% polyacrylamide) of protein lysates prepared from *E. coli* transformed with plasmid pOspA-1 or pHSP70-A encoding spirochetal OspA (rOspA) or HSP70 (rHSP70), respectively, and from the *B. burgdorferi* CA12 isolate (Bb) are shown. Immunoblotting was performed with serum samples from a rabbit immunized with *B. burgdorferi*, from patients (1, 2, and 3) with Lyme arthritis, and from a healthy control. Arrowheads indicate the position of HSP70.

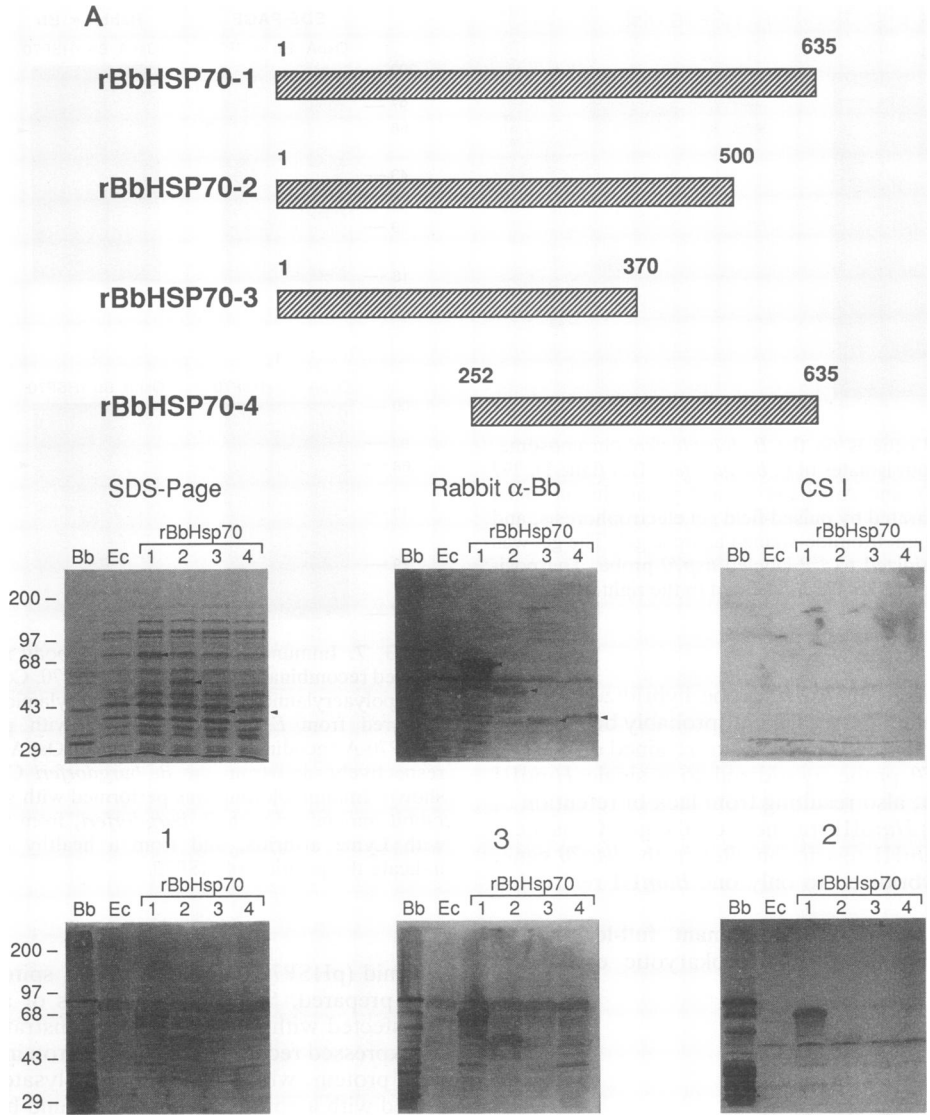

FIG. 8. (A) Diagrammatic representation of the expressed recombinant *B. burgdorferi* HSP70s. The positions of the amino- and carboxy-terminal amino acids of the expressed full-length (BbHSP70-1) and truncated (BbHSP70-2 to BbHSP70-4) recombinant HSP70s are indicated (left and right of the bars). (B) Immunoblot analysis of antibody reactivity with expressed recombinant full-length and truncated *B. burgdorferi* HSP70s. Coomassie blue-stained SDS–10% polyacrylamide gels of protein lysates prepared from the CA12 isolate of *B. burgdorferi* (lanes Bb) or from *E. coli* transformed with control plasmid (lanes Ec), or plasmids encoding full-length (lanes BbHsp70-1) or truncated (lanes BbHsp70-2 to BbHsp70-4) HSP70 proteins, are shown. Immunoblotting was performed with a *B. burgdorferi*-specific rabbit antiserum (rabbit α-Bb), serum from three patients with Lyme arthritis (1, 2, and 3), and serum from a healthy control (CS1). Arrowheads indicate the positions of the expressed rHSP70s. Relative molecular mass markers (in kilodaltons) are indicated to the left.

The binding of antibodies in the serum of Lyme arthritis patients to the truncated rHSP70s was examined by immunoblotting to determine whether antibody-reactive epitopes could be localized to a particular region of the protein (Fig. 8B). Antibodies in the serum of two Lyme arthritis patients (patients 1 and 3) bound to all three truncated rHSP70s. The antibodies in the serum of these patients appear to recognize multiple epitopes on HSP70; confirmation of these epitopes does not require folding of the full-length protein. In contrast, patient 2 had serum antibodies which bound the full-length but not any of the truncated forms of rHSP70. This patient appears to have antibodies recognizing a small number of epitopes on HSP70 and to recognize an epitope(s) whose confirmation is dependent on the folding of the intact protein.

Human T-cell clones isolated from a patient with Lyme arthritis recognize spirochetal rHSP70. Cloned T cells, responding to *B. burgdorferi* antigens, were isolated from the peripheral blood of a patient (patient 1) with chronic Lyme arthritis. Two *B. burgdorferi*-reactive $CD4^+$ $CD8^-$ $TCR\alpha\beta^+$ T-cell clones isolated from this patient recognized rHSP70 expressed in *E. coli* (Fig. 9). These cloned T-cell lines were specific for *B. burgdorferi* HSP70 and did not recognize *E. coli* HSP70, since they did not proliferate in response to a control lysate of *E. coli* proteins. These two T-cell clones also did not recognize other expressed recombinant spiro-

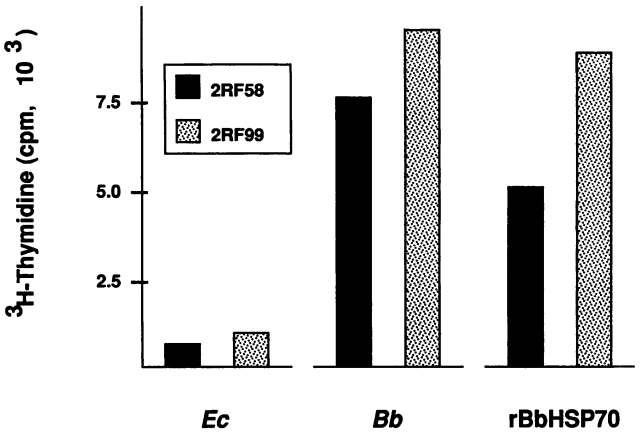

FIG. 9. Human T-cell clones 2RF58 and 2RF99 recognize the expressed rHSP70. The proliferative response of 2×10^4 cells of the indicated T-cell clone after incubation with 4×10^4 autologous B-lymphoblastoid cells and antigen for 72 h is shown. Antigens used in these assays were 1:2,000 dilutions of protein lysates of *B. burgdorferi* (*Bb*) or *E. coli* transformed with either the control plasmid (*Ec*) or the plasmid encoding spirochetal HSP70 (rBbHSP70). The proliferation was determined by measurement of [^{3}H]thymidine incorporation during the last 6 h of incubation. The results presented are the means of triplicate determinations, and the standard deviation for each datum point is less than 10%.

chetal proteins, including OspA, OspB, flagellin, and HSP60 (data not shown). Forty-one other *B. burgdorferi*-reactive T-cell clones isolated from this patient did not recognize the expressed rHSP70 (16a).

Antibodies in the serum of three Lyme arthritis patients bind to *B. burgdorferi* HSP70 but not human HSP70. To determine whether antibodies in the serum of patients with Lyme disease, which recognize a particular spirochetal antigen, also bind the homologous human protein, we used immunoblotting to examine the binding of antibodies in the serum of three patients with Lyme arthritis to HSP70 homologs purified from *E. coli*, *M. tuberculosis*, bovine brain, or a human cell line. Four distinct human HSP70 homologs (HSP70, HSP72, mitochondrial HSP75, and BiP) were purified from a detergent lysate of a human cell (HeLa) line (Fig. 10, lanes Hu^2), while one HSP70 homolog (HSP72) was purified from the soluble cytoplasmic fraction (lanes Hu^1). Although all three Lyme arthritis patients had serum antibodies reactive with the expressed recombinant *B. burgdorferi* HSP70, there was not detectable antibody reactivity with any of the human or bovine brain HSP70 proteins (Fig. 10). However, these patients differed with respect to antibody cross-reactivity with HSP70 homologs of other bacterial species. Antibodies in the serum of one patient (patient 1) reacted with HSP70 purified from *M. tuberculosis* and exhibited weaker binding to *E. coli* HSP70. In contrast, antibodies in the serum of patient 2 did not react with either bacterial HSP70. Antibodies in the serum of patient 3 had only weakly detectable reactivity with *E. coli* HSP70 and no detectable reactivity with *M. tuberculosis* HSP70.

DISCUSSION

In this study, we have isolated and analyzed the gene encoding an immunoreactive *B. burgdorferi* HSP70 homolog. We have demonstrated that this spirochetal gene encodes a member of the HSP70 family of proteins on the

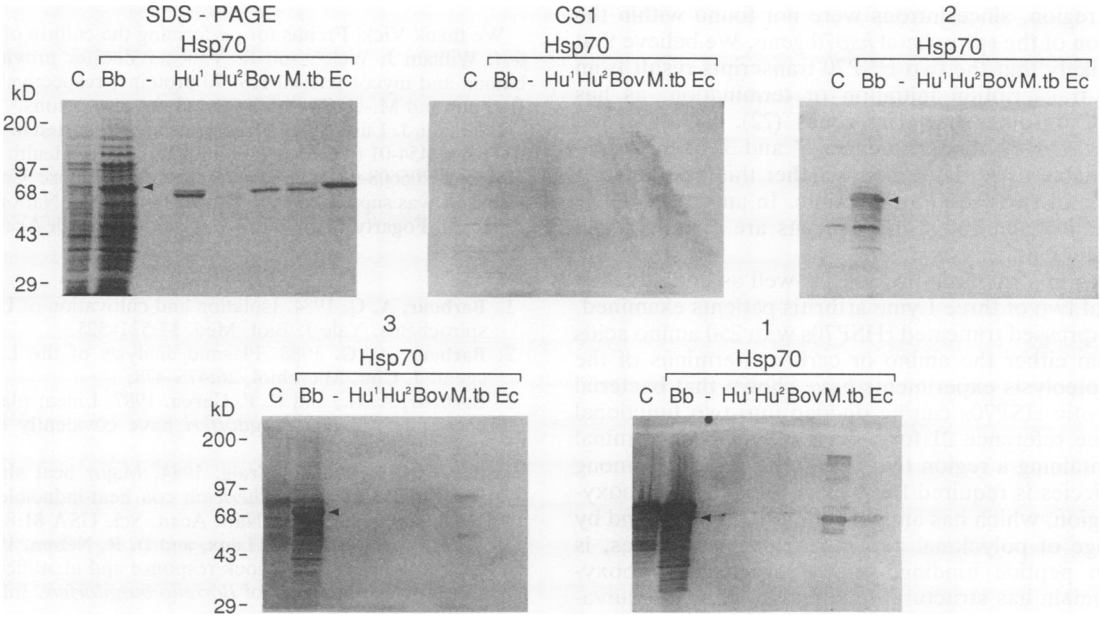

FIG. 10. Immunoblot analysis of antibody reactivity with bacterial and mammalian HSP70. Coomassie blue-stained SDS–10% polyacrylamide gels of a protein lysate prepared from *E. coli* transformed with a control plasmid (lanes C) or plasmid pHSP70-1 encoding *B. burgdorferi* HSP70 (lanes Bb); HSP70 proteins purified from *E. coli* (lanes Ec), *M. tuberculosis* (lanes M.tb.), or bovine brain (lanes Bov); or human HSP70 proteins purified from the soluble cytosolic fraction (lanes Hu^1) or total detergent extract (lanes Hu^2) of HeLa cells are shown. Immunoblotting was performed with serum samples from a healthy control (CS1) and from three patients with Lyme arthritis (1, 2, and 3). Arrowheads indicate the positions of the expressed recombinant *B. burgdorferi* HSP70 homolog. Relative molecular mass markers (in kilodaltons) are indicated to the left.

basis of its amino acid sequence homology with other bacterial and human HSP70s and by the thermal inducibility of its corresponding RNA. The reactivity of the expressed recombinant protein encoded by this gene with antibodies and T cells isolated from patients with Lyme disease provides additional confirmation that this gene encodes the spirochetal HSP70 homolog. The thermoinduction of spirochetal HSP70 RNA transcripts and the lack of thermal inducibility of HSP60 RNA correlate with the results of previous studies examining the thermal inducibility of the synthesis of these spirochetal proteins (5, 6, 17). The synthesis of HSP70 homologs in *B. burgdorferi* is increased at higher temperature, whereas that of HSP60, which is prominently expressed at normal growth temperature, is not.

The mechanism by which the two HSP70 RNA transcripts are generated and their relationship to the 71- and 73-kDa spirochetal HSP70 homologs are unexplained at present. Protein lysates of *B. burgdorferi* contain two HSP70 homologs of 71 and 73 kDa; they have identical NH_2-terminal amino acid sequences but can be resolved by two-dimensional gel electrophoresis (17). Both HSP70 RNA transcripts appear to originate from the single *hsp70* gene found on the spirochetal chromosome. There are a number of different explanations of how the two *B. burgdorferi* HSP70 RNA transcripts and proteins originate. Since the 71- and 73-kDa HSP70s have identical amino-terminal sequences, it is possible that differential posttranslational modification of a single spirochetal protein precursor produces these two proteins. Alternatively, the 71- and 73-kDa proteins may each be derived from translation of a different HSP70 RNA transcript. The two HSP70 RNA transcripts may result from posttranslational processing of HSP70 RNA. Self-splicing group I introns have been found within the genes encoding leucine tRNA in cyanobacteria (16, 31) and DNA polymerase of bacteriophage SPO1, whose host is *Bacillus subtilis* (10). However, RNA processing would have to occur in a noncoding region, since introns were not found within the coding region of the spirochetal *hsp70* gene. We believe that it is more likely that the two HSP70 transcripts result from differential transcription initiation or termination, as has been noted for other bacterial genes (22). Isolation and analysis of the nucleotide sequences 5′ and 3′ of the *hsp70* gene will enable us to determine whether this accounts for the presence of two HSP70 transcripts. In any case, determining which of the above mechanisms are operative will require additional analysis.

Antibodies in a rabbit antiserum, as well as antibodies in the serum of two of three Lyme arthritis patients examined, bound to expressed truncated rHSP70s with 250 amino acids deleted from either the amino or carboxy terminus of the protein. Proteolysis experiments have shown that bacterial and eukaryotic HSP70s can be divided into two functional domains (see reference 21 for a review). An NH_2-terminal domain containing a region that is highly conserved among different species is required for ATP binding. The carboxy-terminal region, which has antigenic determinants bound by a wide range of polyclonal and monoclonal antibodies, is involved in peptide binding. Interestingly, the carboxy-terminal domain has structural features, along with equivalent amino acids at key positions, very similar to those of another group of peptide-binding proteins, human leukocyte antigen class I proteins (23). Therefore, the increased divergence of the COOH-terminal sequence of spirochetal HSP70 from that of the other HSP70s is consistent with the known properties of this domain. This domain of spirochetal HSP70 may contain nonconserved epitopes recognized by antibodies in the serum of patients with Lyme disease. Therefore, it is likely that the specificity of serologic tests for the diagnosis of Lyme disease can be improved through the use of synthetic peptides or truncated recombinant proteins corresponding to the nonconserved carboxy-terminal region of spirochetal HSP70.

The presence of T cells and antibodies reactive with spirochetal HSP70 in patients with Lyme arthritis is consistent with the fact that the HSP70 homologs of several different infectious pathogens are immunodominant antigens (see references 25 and 32 for reviews). The reasons why HSP70 homologs are immunodominant antigens and what role, if any, the immune response to these antigens plays in the pathogenesis of human inflammatory diseases are unknown at present. The isolation and characterization of the spirochetal HSP70 homolog will allow these questions to be investigated by using Lyme disease as a model system. To directly investigate the possibility that antigenic mimicry plays a role in the pathogenesis of inflammatory arthritis, we performed immunoblotting with serum samples obtained from patients with Lyme arthritis. Although antibodies in the serum of three Lyme arthritis patients bound to *B. burgdorferi* HSP70, there was no detectable binding to human HSP70 homologs. This decreases the likelihood that cross-reactive antibodies recognizing both pathogen and host HSP70 proteins significantly contribute to the pathogenesis of inflammatory arthritis in these patients. However, this possibility is not completely excluded by this preliminary analysis of a small number of patients. Characterization of HSP70-reactive antibodies and T-cell clones obtained from additional patients with Lyme disease, which is facilitated by the availability of expressed rHSP70, will be required to determine what role, if any, the immune response to spirochetal HSP70 plays in the pathogenesis of Lyme disease.

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