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Characterization of the Heat Shock Response and Identification of Heat Shock Protein Antigens of *Borrelia burgdorferi*

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The heat shock response of *Borrelia burgdorferi* B31 cells was characterized with regard to the heat shock proteins (Hsps) produced. Five to seven Hsps were detected by sodium dodecyl sulfate-gel electrophoresis and fluorography of proteins from cells labeled with [^{35}S]methionine after shifts from 33°C to 37 or 40°C or from 20°C to 33, 37, or 40°C. Analysis of [^{35}S]methionine-labeled Hsps by two-dimensional electrophoresis and autoradiography revealed 12 Hsps. Western immunoblot analysis with antisera to highly conserved *Escherichia coli* and *Mycobacterium tuberculosis* Hsps revealed a single 72-kilodalton (kDa) protein band that reacted with antibodies to *E. coli* DnaK and with antibodies to the *M. tuberculosis* 71-kDa Hsp homolog of *E. coli* DnaK. Two proteins with apparent molecular masses of 66 and 60 kDa reacted with antibodies against the *M. tuberculosis* 65-kDa Hsp homolog of *E. coli* GroEL. Human immune sera collected from patients with Lyme disease reacted with both the 66-kDa Hsp and the 60-kDa Hsp but failed to react with the 72-kDa Hsp. These data are discussed with regard to the possibility that host recognition of highly conserved epitopes of GroEL homologs of *B. burgdorferi* may result in autoimmune reactions causing arthritis and other pathologies.

Borrelia burgdorferi was shown to be the causative agent of Lyme disease in 1982 (4, 5, 30). Cases of this tick-borne bacterial infection are widely found in North America, Europe, and Asia and are reported to be increasing in both number and geographic distribution (3). Lyme disease exhibits a broad range of clinical features, usually in three progressive stages (1, 10). In stage I, the earliest manifestation is the characteristic expanding annular rash, erythema migrans, around the tick bite. This may be followed by a variety of symptoms including headache, malaise, fatigue, low-grade fever, and regional lymphadenopathy. Stage II symptoms may include pathological changes in the heart and meningitis. Stage III is often characterized by arthritis and various neuropathies. It has been suggested that many stage III symptoms may have an autoimmune origin (10, 29).

The intent of this investigation was to examine the heat shock response of *B. burgdorferi* to identify the heat shock proteins (Hsps) produced by this pathogenic bacterium and to determine whether any of these proteins was recognized as an antigen by the human immune system. All organisms respond to elevated temperature by altering their pattern of growth and protein synthesis (23, 25). Upon exposure to elevated temperatures, cells rapidly cease growth, repress the synthesis of many vegetative proteins, and coordinately and preferentially synthesize a small number of highly conserved proteins (23, 25). This is termed the heat shock response.

Recently, several (five to six) major antigenic proteins of *Mycobacterium leprae* and *Mycobacterium tuberculosis* were shown to be Hsps (35, 36). These antigenic Hsps include a 65-kilodalton (kDa) protein and a 71-kDa protein, homologs of *Escherichia coli* GroEL and DnaK, respectively (35, 36). Additionally, a 60-kDa antigen of *Legionella pneumophila* (17) and a 62-kDa antigen of *Coxiella burnetti* (33) have been identified as Hsps and are believed to be homologous to *E. coli* GroEL. Previous studies of *B. burgdorferi* have identified a number of major antigens. These include the OspA and OspB outer surface proteins (31 and 34 kDa, respectively), the 41-kDa flagellin, the 21- to 22-kDa pC antigen, and the 60-kDa common antigen (3, 13). It has been suggested that this last protein may be a homolog of *E. coli* GroEL and thus may be an Hsp (13).

In this paper, we characterize the heat shock response of *B. burgdorferi* and demonstrate that at least 12 Hsps are synthesized by this microbe. Additionally, Hsps homologous to *E. coli* DnaK and GroEL were identified in *B. burgdorferi*. Identifications were based on immunological reactivity, molecular weight, and induction by heat shock. One DnaK-like protein and two GroEL-like proteins were observed. Further, the proteins homologous to GroEL reacted with human immune sera from Lyme disease patients.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Cultures of *B. burgdorferi* B31 (ATCC 35210) were maintained on modified BSK II medium (2) at either 20 or 33°C.

Heat shock procedure and radioactive labeling of cell proteins. Cultures used for heat shock experiments were grown at 33°C for 3 to 4 days or at 20°C for 10 to 12 days. Cells were removed from BSK II medium by centrifugation at 9,000 × *g* for 10 min and suspended in labeling medium lacking methionine (RPMI 1640 Selectamine medium; GIBCO Laboratories, Grand Island, N.Y.). After two additional centrifugation and resuspension steps, 1-ml samples of the concentrated cell suspension were pipetted into microfuge tubes and equilibrated for 10 min at either 20 or 33°C, depending on the nature of the experiment. Some tubes were then transferred to higher temperatures, whereas other tubes were left at the control temperatures of 20 and 33°C. The temperature shifts used to characterize the heat shock response of *B. burgdorferi* were: 20°C to 33°C, 20°C to 37°C, 20°C to 40°C, 33°C to 37°C, and 33°C to 40°C. These temperatures were selected for the following reasons: (i) 20°C represents an ambient temperature that *B. burgdorferi* cells might experience in the tick vector; (ii) 33°C is a near-optimal growth temperature and represents normal human skin temperature;

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(iii) 37°C is the normal human body temperature and is a supraoptimal growth temperature, which should transiently induce the heat shock response; and (iv) for many organisms 40°C is a lethal temperature that should induce the heat shock response and also simulate a fever temperature, which may affect the host-parasite relationship. After a 10-min equilibration period at higher temperatures, all cultures were labeled with 10 μl of [^{35}S]methionine (1 μCi/μl; specific activity, >600 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.) and incubated for an additional 60 min. The labeling period was terminated by placing cultures on ice and removing cells from the labeling medium by centrifugation at 4°C (15,000 × *g* for 2 min). Cells were then washed twice in 1 ml of 10 mM Tris buffer (pH 7.6) at 0°C. Cell pellets were suspended in 50 μl of solubilization-reduction mixture (21) in preparation for gel electrophoresis. These samples were frozen and stored at −20°C.

Electrophoretic methods. One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 0.75-mm-thick slab gels (5% polyacrylamide stacking gel, 12.5% polyacrylamide separation gel) as described by Laemmli (21). Gel lanes were loaded with equal radioactive counts. Gels were fixed in a solution containing 25% isopropanol and 10% acetic acid for at least 1 h and prepared for fluorography with Fluoro-Hance (Research Products International Corp., Mount Prospect, Ill.) according to the instructions of the manufacturer. Proteins were visualized by exposing Kodak X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.) to the dried gel at −70°C. Two-dimensional gel electrophoresis was performed by the method of O'Farrell (27). Proteins were separated in the first dimension by isoelectric focusing in a pH 3 to 10 gradient followed by SDS-PAGE in 10 to 20% polyacrylamide slab gels. Proteins were visualized by autoradiography.

Western blot procedure. Cultures used in the Western immunoblot procedure were grown and heat shocked as described above. In some experiments cultures were labeled with [^{35}S]methionine as described above. After centrifugation and rinsing, cells were suspended in 50 μl of 10 mM Tris buffer (pH 7.5) and frozen at −20°C. Protein concentrations of each sample were determined by using Bradford reagent (Bio-Rad Laboratories, Richmond, Calif.) so gels could be loaded with equal amounts of protein (15 to 20 μg) per lane. Proteins were separated by SDS-PAGE and transferred to nitrocellulose with a transblot apparatus (Bio-Rad) run at 30 V for 3 to 7 h by the procedure of Towbin et al. (31). Nitrocellulose was placed overnight in blocking buffer (phosphate-buffered saline, 0.05% Tween 20, 1% bovine serum albumin) and rinsed three times in washing buffer (phosphate-buffered saline–0.05% Tween 20). Nitrocellulose strips were then placed in human or rabbit test sera (see below) and incubated for 1 h at room temperature. After three washes in washing buffer, the strips were transferred to trays containing peroxidase-labeled anti-immunoglobulin. The anti-immunoglobulin employed was either affinity chromatography-purified peroxidase-labeled goat anti-human immunoglobulin G (Sigma Chemical Co., St. Louis, Mo.) diluted 1:250 in washing buffer or affinity chromatography-purified peroxidase-labeled goat anti-rabbit immunoglobulin G (Sigma) diluted 1:250 in washing buffer. After 1 h of incubation at room temperature, the strips were rinsed three times with phosphate-buffered saline without Tween 20, and reactive proteins were detected by using the color developer 3,3′-diaminobenzidine tetrahydrochloride (Sigma) according to the instructions of the manufacturer. Strips were then rinsed in distilled water, dried, and mounted on Whatman filter paper. In some cases, X-Omat AR film was exposed to blots containing [^{35}S]methionine-labeled proteins to facilitate identification of antigenic proteins. The autoradiogram was superimposed over the immunoblot to confirm the identity of specific antigenic proteins.

The human immune sera employed in the Western blot analysis were obtained from patients diagnosed as having Lyme disease and were generously provided by R. Ryan, University of Connecticut Health Center, Farmington. Diagnosis of Lyme disease in these patients was based on clinical history, clinical symptoms, and titers against *B. burgdorferi* in serum. Each of the 10 sera employed had an immunoglobulin G titer of 1:>1,280 in a solid-phase enzyme-linked immunosorbent assay (24). Control human sera were obtained from seven healthy volunteer donors with no history of spirochete infection. All sera were diluted in washing buffer and employed at dilutions of 1:40 to 1:100.

Rabbit polyclonal antiserum prepared against purified *E. coli* DnaK was generously provided by C. Georgopoulos, University of Utah. Rabbit polyclonal antisera prepared against purified *M. tuberculosis* Hsp71 and Hsp65 were generously provided by A. Mehlert and D. Young, University of London. These antisera were diluted in washing buffer and employed at a 1:1,000 dilution.

***Borrelia* 66-kDa protein.** A 66-kDa protein isolated from *B. burgdorferi* by the method of Hendrix (generously provided by R. Fister and R. Ryan, University of Connecticut Health Center) (15) was subjected to SDS-PAGE and transferred to nitrocellulose paper as described above. Potential reactivity with human sera (diluted 1:40 with washing buffer) from healthy persons and Lyme disease patients and with antibodies raised against *M. tuberculosis* Hsp65 or Hsp71 (diluted 1:1,000) was tested by the immunoblot procedure described above.

Immunoprecipitation. Immunoprecipitation of *B. burgdorferi* proteins with sera from healthy persons and Lyme disease patients as well as antiserum to *M. tuberculosis* Hsp65 was performed by the protocol of Ito et al. (19). Briefly, *B. burgdorferi* cells were labeled with [^{35}S]methionine at 33 or 40°C as described above and then precipitated with cold 10% trichloroacetic acid. The trichloroacetic acid precipitates were washed in acetone and dissolved in 25 μl of 1% SDS–50 mM Tris hydrochloride (pH 8)–1 mM EDTA. Samples were then diluted 1:33 in cold Triton buffer (2% Triton X-100, 50 mM Tris hydrochloride [pH 8], 0.15 M NaCl, 0.1 mM EDTA). Equal amounts of ^{35}S-labeled material were then mixed with either 25 μl of undiluted human immune serum or 10 μl of undiluted rabbit anti-*M. tuberculosis* Hsp65. After 16 h of incubation of 4°C, the mixtures were immunoprecipitated by the addition of 50 μl of Formalin-fixed *Staphylococcus aureus* protein A-bearing cells (Sigma). Immunoprecipitates were analyzed by SDS-PAGE and fluorography as described above.

RESULTS

Production of Hsps in cells grown at 33°C. To test the hypothesis that some *B. burgdorferi* Hsps are antigens that are recognized by the infected host, Hsps produced by *B. burgdorferi* had to be identified. Cells were pulse-labeled with [^{35}S]methionine at the growth temperature (33°C) or at the heat shock temperature (37 or 40°C), and the proteins analyzed by SDS-PAGE and fluorography. The results of one such experiment are presented in Fig. 1A. Typically, when cells were shifted from 33°C to either 37 or 40°C, five to eight Hsps were detected. The most prominent of these

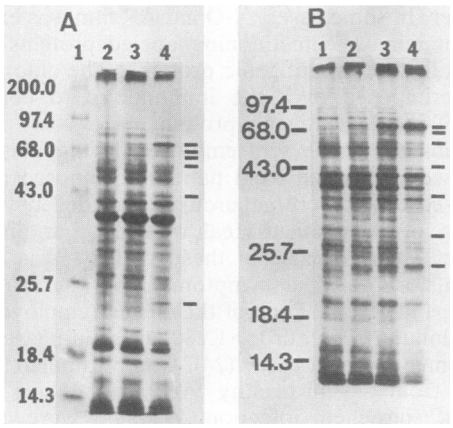

FIG. 1. Fluorograms of Hsps produced by *B. burgdorferi* B-31 cells grown at 33°C (A) or at 20°C (B). (A) Samples (1 ml) of cells (10^9/ml) grown at 33°C were incubated and labeled with [^{35}S]methionine (10 μCi) for 1 h at 33°C (lane 2), 37°C (lane 3), or 40°C (lane 4). Molecular weight standards are shown in lane 1. (B) Samples (1 ml) of cells (10^9/ml) grown at 20°C were incubated and labeled with [^{35}S]methionine (10 μCi) for 1 h at 20°C (lane 1), 33°C (lane 2), 37°C (lane 3), or 40°C (lane 4). Total cell proteins were separated on SDS–12.5% acrylamide gels (21). Equal counts per minute were loaded onto each lane. Hsps are indicated by the lines at the right of each fluorogram.

Hsps were five proteins with estimated molecular masses of 72, 66, 60, 43, and 24 kDa. Additional minor Hsps with approximate molecular masses of 64, 35, and 28 kDa were usually detected. It was thought that a larger shift up in temperature might more fully induce the heat shock response and possibly reveal additional Hsps. To test this possibility, *B. burgdorferi* cells were grown at 20°C and pulse-labeled at 20, 33, 37, or 40°C with [^{35}S]methionine. Analysis of the [^{35}S]methionine-labeled proteins by SDS-PAGE (Fig. 1B), however, showed that essentially the same Hsps were induced by temperature shifts from 20°C to 33, 37, or 40°C as were detected in shifts from 33°C to 37 or 40°C. Specifically, Hsps with apparent molecular masses of 72, 66, 60, 43, 35, 28, and 24 kDa were identified.

Two-dimensional gel analysis of Hsps. To more carefully identify the Hsps produced by *B. burgdorferi*, [^{35}S]methionine-labeled proteins from cells grown at 33°C and pulse-labeled at 33 or 40°C were analyzed by two-dimensional gel electrophoresis (27) and autoradiography (Fig. 2). At least 12 proteins whose expression was enhanced by heat shock were detected. There were also a large number of proteins whose expression was reduced by heat shock. Additionally, the apparent molecular weights of the major Hsps detected in two dimensions corresponded to those identified in one dimension.

Identification of *B. burgdorferi* proteins homologous to *E. coli* DnaK and GroEL. The identification of *B. burgdorferi* Hsps of 72 and 66 kDa suggested the possibility that these proteins are homologs of *E. coli* DnaK and GroEL, respectively. To test this possibility, Western blot analysis was performed on whole-cell extracts of *B. burgdorferi* cells grown at 33°C and heat shocked at 40°C (Fig. 3). Antiserum against *E. coli* DnaK and antiserum against *M. tuberculosis* Hsp71 (DnaK homolog) reacted with a single protein band at 72 kDa. Antiserum against the *M. tuberculosis* Hsp65 (GroEL homolog) reacted with two protein bands at 66 and 60 kDa. Additionally, a sample of a 66-kDa protein purified from *B. burgdorferi* was analyzed by Western blotting. The

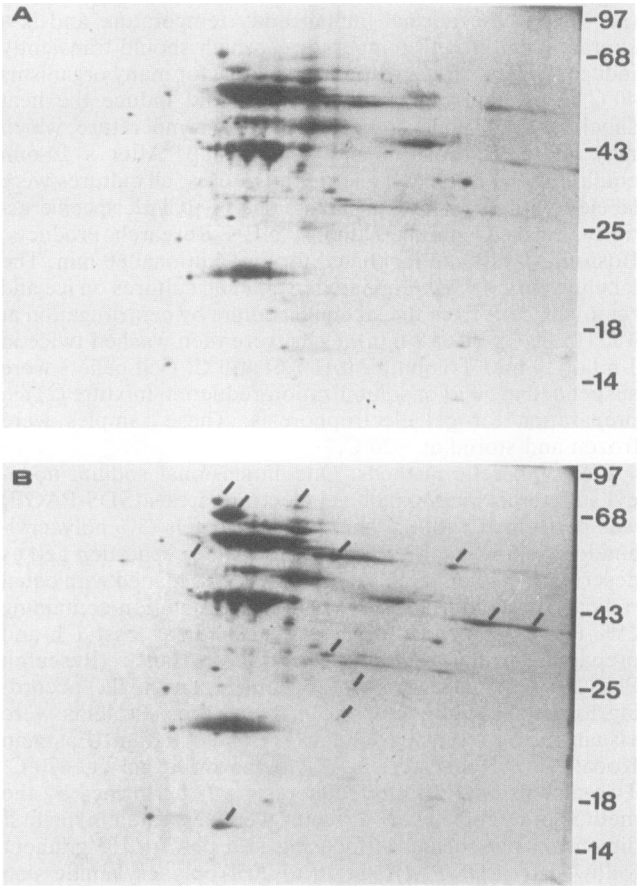

FIG. 2. Autoradiograms of two-dimensional gels showing Hsps produced by heat-shocked *B. burgdorferi* B-31 cells. Cells grown at 33°C were incubated and labeled with [^{35}S]methionine (10 μCi/ml) for 1 h at 33°C (A) or 40°C (B). Total cell proteins were separated by isoelectric focusing in the first dimension (pH 3 to 10, positive pole on the right) and by SDS-PAGE in the second dimension (27). Equal counts per minute were loaded onto each gel, and each gel was exposed for 67.5 h. Hsps are indicated in panel B. Positions of molecular weight standards are indicated on the right.

protein reacted specifically with antiserum to *M. tuberculosis* Hsp65 but not with antibodies to *E. coli* DnaK (Fig. 3) or with antiserum to *M. tuberculosis* Hsp71 (data not shown). That anti-*M. tuberculosis* Hsp65 reacted with two proteins from whole-cell extracts was unusual and suggests the possibility that there are two proteins homologous to GroEL in *B. burgdorferi*.

Hsps react with human immune sera. Determinations of whether any *B. burgdorferi* Hsps are antigens in infected Lyme disease patients were carried out by a combination of Western blot analysis and autoradiography. Total cell proteins from *B. burgdorferi* cells grown at 33°C and labeled with [^{35}S]methionine at 33°C or heat shocked and labeled with [^{35}S]methionine at 40°C were separated by SDS-PAGE, blotted onto nitrocellulose, and reacted with individual human immune or control sera. The resulting blots were then used for autoradiography. The results of one such experiment with human immune serum are shown in Fig. 4. The patterns of immunoreactive bands observed were similar to those reported by other investigators (7, 8), with immunoreaction bands appearing at 85, 66, 60, 43, 41, 35, 33, and 20 kDa. In all cases the reactivity of immune sera tested against

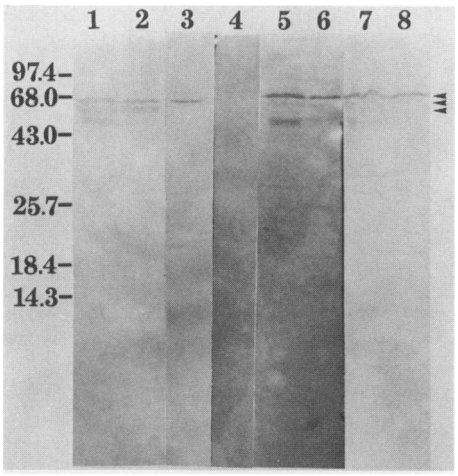

FIG. 3. Identification of *B. burgdorferi* proteins homologous to *E. coli* GroEL and DnaK by Western blot analysis. Proteins from *B. burgdorferi* cells grown at 33°C and heat shocked at 40°C were separated by SDS-PAGE (21) and analyzed by Western blotting (31). Lanes contain proteins from heat-shocked cells (lane 1) and control cells (lane 2) probed with anti-*M. tuberculosis* Hsp65 antibodies, purified *B. burgdorferi* 66-kDa protein probed with anti-*M. tuberculosis* Hsp65 antibodies (lane 3) and anti-*E. coli* DnaK antibodies (lane 4), protein from heat-shocked (lane 5) and control cells (lane 6) probed with anti-*E. coli* DnaK antibodies, and proteins from heat-shocked (lane 7) and control cells (lane 8) probed with anti-*M. tuberculosis* Hsp 71 antibodies. Molecular mass standards in kilodaltons are marked on the left. The arrows on the right indicate the locations of the 72-, 66-, and 60-kDa antigens.

heat-shocked versus non-heat-shocked preparations appeared to be identical. Although minor differences in the patterns of immunoreactive bands were observed when sera from 10 different individuals were employed, the basic pattern was as shown in Fig. 4. All of the immune sera tested reacted with the 66- and 60-kDa Hsp bands. None of the sera tested reacted with the prominent Hsps of 72 or 24 kDa. In some cases, one of the immunoreactive bands (43 kDa) may correspond to what may be a 43-kDa minor Hsp; however, this requires additional experimentation. None of the normal human sera tested showed reactivity against any of the *B. burgdorferi* proteins (data not shown).

Immunoprecipitation of Hsps. Whole-cell extracts of heat-shocked and control *B. burgdorferi* cells pulse-labeled with [^{35}S]methionine were immunoprecipitated with human immune serum and compared with extracts immunoprecipitated with antiserum against *M. tuberculosis* Hsp65 (Fig. 5). Immunoprecipitation with human immune serum resulted in the recovery of a number of immunoreactive proteins, the most prominent of which was a 66-kDa protein. Similarly, when anti-*M. tuberculosis* Hsp65 was employed, the principle protein detected was a 66-kDa protein that comigrated with the principal protein precipitated with human immune serum. Several additional proteins could be seen in both preparations. In the case of anti-*M. tuberculosis* Hsp65, these were not detected in the Western blot assay (Fig. 3), so it seems likely that either these proteins were passively trapped in the precipitated material, or these proteins may have interacted with the GroEL homolog and coprecipitated with the GroEL homolog because of the possible chaperonin functions of the protein (6, 9, 11, 14). Alternatively, they may simply represent breakdown products of the GroEL homolog.

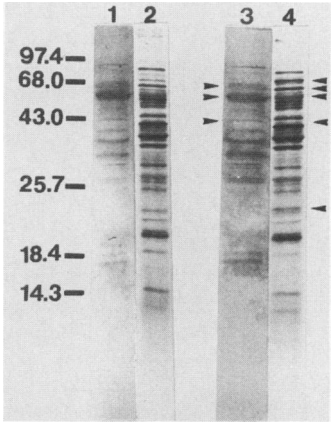

FIG. 4. Identification of *B. burgdorferi* Hsps recognized by human immune serum from Lyme disease patients. Samples of cells grown at 33°C were incubated and labeled with [^{35}S]methionine (10 μCi/ml) for 1 h at 33°C (lanes 1 and 2) or 40°C (lanes 3 and 4). Total cell proteins were separated by SDS-PAGE (12.5% acrylamide gels) (21) and transferred to nitrocellulose (31). The nitrocellulose strips were probed with human immune serum from a Lyme disease patient (titer of 1:5,120) and the immunoreactive bands were visualized with goat anti-human immunoglobulin G antibodies conjugated to horseradish peroxidase (lanes 1 and 3). The strips were then used for autoradiography (lanes 2 and 4). The major Hsps visualized in this experiment are marked on the right, and Hsps reacting with human immune serum are marked on the left. Equal counts per minute were loaded onto lanes 2 and 4.

DISCUSSION

All organisms respond to elevations in temperature by temporarily altering their pattern of protein synthesis and growth (23, 25). This response has been termed the heat shock response. Recently, a number of investigators have suggested that several Hsps may be involved in the immunology and pathology of mycobacterial diseases (32, 33).

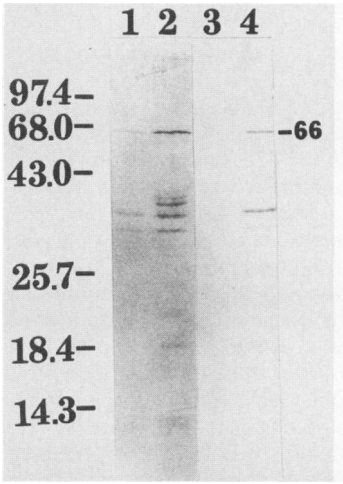

FIG. 5. Fluorogram of proteins immunoprecipitated from *B. burgdorferi* cells. Cultures were grown at 33°C, and samples were labeled for 1 h with [^{35}S]methionine (10 μCi/ml) at 33°C (lanes 1 and 3) or at 40°C (lanes 2 and 4). Proteins were reacted with human immune serum (lanes 1 and 2) or rabbit anti-*M. tuberculosis* Hsp65 (lanes 3 and 4) and immunoprecipitated with *S. aureus* bearing protein A (19). Immunoprecipitated proteins were separated on an SDS–12.5% acrylamide gel.

Two Hsps in particular, Hsp71 and Hsp65 (DnaK and GroEL homologs, respectively), have been shown to be major antigens in *M. tuberculosis* and *M. leprae* (35, 36). These two immunodominant Hsps appear to be involved in both humoral and cellular immunity (19, 26, 29). Additionally, it has been suggested that antibodies to these proteins may react with homologous host proteins to produce autoimmune pathologies such as arthritis (20, 28). These observations argue that a similar situation may exist in Lyme disease (13).

The data presented in this study demonstrate that *B. burgdorferi* exhibits a heat shock response when it experiences an elevation in temperature. Elevations in temperature that trigger the heat shock response may be small (i.e., 33°C to 37°C) or large (i.e., 20°C to 40°C); also, triggering temperature elevations may involved shifts to supraoptimal temperatures (i.e., shifts to 37 or 40°C) or shifts from low to optimal growth temperatures (i.e., 20°C to 33°C). In all respects, these results are similar to those documented for other bacteria (12, 25, 26). Also, as is the case with other systems, many of these proteins are present before the shift in temperature but are preferentially synthesized immediately after the shift in temperature.

In *B. burgdorferi*, the heat shock response involves the increased or de novo expression of at least 12 proteins detected by two-dimensional gel electrophoresis of heat-shocked cell extracts labeled with [^{35}S]methionine. It is possible that additional Hsps may exist but are not detected when cells are labeled with [^{35}S]methionine. Labeling cells with ^{14}C- or ^{3}H-amino acids could reveal additional Hsps in *B. burgdorferi*. However, *B. burgdorferi* cells do produce proteins homologous to DnaK and GroEL, the most highly conserved of the Hsps. These Hsps were identified by three criteria: (i) induction by heat shock, (ii) apparent molecular weight by SDS-PAGE, and (iii) reactivity with antibodies against *E. coli* DnaK and GroEL.

The DnaK homolog of *B. burgdorferi* is a 72-kDa protein that reacts with both anti-*E. coli* DnaK antibodies and anti-*M. tuberculosis* Hsp71 antibodies. By one-dimensional SDS-PAGE this protein appears to be the most strongly induced Hsp in *B. burgdorferi* cells. Analysis of Hsps by two-dimensional gel electrophoresis also shows that this DnaK homolog is the most strongly induced and abundant Hsp. Additionally, two other Hsps with slightly greater molecular weights, not seen by one-dimensional SDS-PAGE, can be detected. It is possible that these two proteins comigrate with the 72-kDa Hsp in one-dimensional SDS gels and could therefore represent members of a 70-kDa Hsp gene family. Although such gene families are common in eucaryotes, they are unusual in procaryotes (23). If these proteins are in fact members of a 70-kDa Hsp gene family, then it could be predicted that they should possess the ability to bind ATP as do other DnaK homologs (23, 34).

Surprisingly, there appear to be two GroEL homologs produced by *B. burgdorferi*. The apparent molecular masses of these proteins are 66 and 60 kDa. Each protein is heat shock inducible and reacts with anti-*M. tuberculosis* Hsp65 antibodies. Whether the apparent presence of two GroEL homologs indicates the presence of two distinct genes or whether the 60-kDa protein is a breakdown product or a processed form of the 66-kDa protein remains to be determined.

Perhaps the most interesting observations about the Hsps of *B. burgdorferi* concern the potential of some of those proteins to serve as antigens during the course of the infection. The results of Western blot analysis of whole-cell lysates of *B. burgdorferi* probed with human immune sera demonstrate that a large number of proteins are antigenic in humans. This corresponds to what has been documented by other groups (7, 8). In this report, we demonstrate that at least two of the proteins (66 and 60 kDa) that react with human immune sera correspond in molecular mass to the *B. burgdorferi* Hsps. Furthermore, the 66-kDa protein could be precipitated by either human immune serum or antiserum against the *M. tuberculosis* GroEL homolog. At present it is not clear why the 60-kDa protein was not detected by the immunoprecipitation procedure; however, it seems likely that this was due to the fact that the 60-kDa protein appears to label less intensely than the 66-kDa protein and appears to produce a much more diffuse electrophoretic band.

Together, these results strongly suggest that Hsp66 and Hsp60 of *B. burgdorferi*, like GroEL homologs in other pathogens, are major antigens (16, 17, 33, 35, 36). In addition, the data provide support for the hypothesis that the common antigen of *B. burgdorferi* is indeed an Hsp (13). These findings are important for at least two reasons. First, the immune response to these highly conserved Hsps, particularly to GroEL homologs, has been implicated in development of autoimmune pathologies such as arthritis (18, 20, 22). It should also be noted that it has been suggested that proteins comprising the Hsp70-DnaK group may have the same potential as autoimmunogens as the Hsp65-GroEL proteins (20). We could find no evidence that the 72-kDa *B. burgdorferi* DnaK homolog is an antigen in the human host, since immune serum from the Lyme disease patients used in this study consistently failed to react with this protein. This is noteworthy because the 72-kDa Hsp is the most heavily expressed Hsp of *B. burgdorferi* during heat shock. However, the lack of immunoreactivity should be viewed cautiously, because only a limited number of sera were examined.

Second, if it is true that the highly conserved GroEL-like proteins of *B. burgdorferi* act as autoimmunogens, then it may also be true that these proteins have the potential to serve as protective immunogens. In the *Mycobacterium* system, Holoshitz et al. (18) have described two T-cell clones that recognize the 65-kDa Hsp of *M. tuberculosis*. One clone is arthritogenic and capable of passively transferring adjuvant-induced arthritis to nonimmune animals, whereas the other confers resistance to *M. tuberculosis*-induced adjuvant arthritis. Together the data suggest, and it seems likely, that the GroEL-like and DnaK-like proteins contain both some highly conserved epitopes and some unique epitopes. Thus immunological recognition of such proteins may be a double-edged sword; some epitopes may confer protection to the host, whereas recognition of other epitopes may lead to autoimmunity.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

Several Hsps identified in this study correspond closely in apparent molecular weight to the four Hsps identified by

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