Cloning and Sequence of the Gene for Heat Shock Protein 60 from *Chlamydia trachomatis* and Immunological Reactivity of the Protein

MICHAEL C. CERRONE,[1] JEFFREY J. MA,[1] AND RICHARD S. STEPHENS[1,2,3]*

Departments of Pharmaceutical Chemistry[1] and Laboratory Medicine[2] and the Francis I. Proctor Foundation,[3] University of California-San Francisco, San Francisco, California 94143-0412

We isolated and sequenced the gene for the chlamydial heat shock protein 60 (HSP-60) from a *Chlamydia trachomatis* genomic library by molecular genetic methods. The DNA sequence derived revealed an operon-like gene structure with two open reading frames encoding an 11,122- and a 57,956-Da protein. The translated amino acid sequence of the larger open reading frame showed a high degree of homology with known sequences for HSP-60 from several bacterial species as well as with plant and human sequences. By using the determined nucleotide sequence, fragments of the gene were cloned into the plasmid vector pGEX for expression as fusion proteins consisting of glutathione S-transferase and peptide portions of the chlamydial HSP-60. HSP-60 antigenic identity was confirmed by an immunoblot with anti-HSP-60 rabbit serum. Sera from patients that exhibited both high antichlamydial titers and reactivity to chlamydial HSP-60 showed reactivity on immunoblots to two fusion proteins that represented portions of the carboxyl-terminal half of the molecule, whereas fusion proteins defining the amino-terminal half were nonreactive. No reactivity with the fusion proteins was seen with sera from patients that had been previously screened as nonreactive to native chlamydial HSP-60 but which had high antichlamydial titers. Sera from noninfected control subjects also exhibited no reactivity. Definition of recognized HSP-60 epitopes may provide a predictive screen for those patients with *C. trachomatis* infections who may develop damaging sequelae, as well as providing tools for the study of immunopathogenic mechanisms of *Chlamydia*-induced disease.

Chlamydia trachomatis is an obligate intracellular bacterium that parasitizes eucaryotic cells and has a unique growth cycle involving the conversion between two distinct developmental forms. The intracellular reticulate body (RB) is metabolically active, has a fragile outer membrane, and replicates by binary fission. The elementary body (EB) is a metabolically inert developmental form that has a rigid, osmotically resistant outer membrane. The outer membrane organization is unique to chlamydiae and is composed of three quantitatively predominant cysteine-rich proteins. These cysteine-rich proteins form extensive disulfide cross-links within the outer membrane, providing structural rigidity for the EB outer membrane in the absence of a peptidoglycan layer (39). Conversion between the two developmental forms during the life cycle requires the assembly, disassembly, and rearrangement of these outer membrane proteins. The molecular processes by which this unique reorganization occurs are not understood.

C. trachomatis is an important etiologic agent of ocular and urogenital tract infections, resulting in both significant monetary cost and high reproductive and ophthalmological morbidity (32). Human genital tract infection with *C. trachomatis* may result in urethritis, epididymitis, salpingitis, and endometritis, with negative long-term outcomes including infertility and ectopic pregnancy. Ocular infections with *C. trachomatis* are the leading cause of preventable blindness in developing nations with endemic trachoma. Disease results from an aggressive inflammatory response that causes tissue injury followed by scarring (15). The severity of the inflammatory response increases with persistent infection or frequent reinfection (15). In addition to the immunopathology induced at the site of infection, there can be systemic

sequelae at distant sites. Several studies on the incidence of sexually acquired reactive arthritis have reported acute *C. trachomatis* infections in 42 to 69% of individuals immediately preceding the onset of arthritis (22, 25, 58). In one study, Ghinsberg et al. (13) found that ~60% of patients characterized as having rheumatoid factor and autoimmune disease were infected with *C. trachomatis*.

A chlamydial component present in the Triton X-100-soluble fraction of chlamydia EBs has been reported to induce ocular inflammatory responses in previously infected animals (54, 62). The predominant antigenic component present in the Triton X-100-soluble fraction was shown to be a protein of approximately 57,000 molecular weight (36), and when partially purified it is capable of eliciting ocular hypersensitivity reactions in guinea pigs previously infected with *Chlamydia psittaci* (36). The Triton X-100-solubilized protein is serologically related to proteins of approximately 60,000 molecular weight found in many gram-negative bacteria, referred to as common antigen (51, 52). Wagar et al. (60) demonstrated that the ~60,000-molecular-weight protein obtained following Triton X-100 treatment of *C. trachomatis* EBs is serologically related to the heat shock protein 60 (HSP-60) family. Bavoil et al. (4) found that the Triton X-100-soluble ~60,000-molecular-weight protein of *C. trachomatis* was antigenically identical to the HSP-60 family and contains reactive sulfhydryl residues. Although most of this protein is recoverable from detergent treatments of EBs, some of the protein is found associated with the outer membrane complex. Recently, Morrison et al. (35) cloned and sequenced the HSP-60 gene for *C. psittaci* guinea pig inclusion conjunctivitis. Heat shock proteins of ~60,000 molecular weight, or HSP-60s, are one class of phylogenetically highly conserved proteins that are induced to high levels of expression in stressed cells but are also expressed at lower levels in nonstressed cells (29). They appear to

---

* Corresponding author.

function in aiding nascent protein chains to assume proper conformational folding during synthesis (6), protein refolding following translocation through membranes (9), and the assembly of multimeric protein complexes (14, 44).

Analysis of both the role that the *C. trachomatis* HSP-60 may play in the induction of either immunopathological or protective immune responses and its role as a potential component of the cellular outer membrane reorganization mechanisms that are part of the unique chlamydial development cycle would be facilitated if the sequence of the HSP-60 were known and if recombinant antigens were available. To study these issues, we have cloned and sequenced the gene encoding the *C. trachomatis* HSP-60 homolog (Chl-GroEL), as well as that encoding the homolog of a second, smaller heat shock protein, GroES. Furthermore, we have defined several antigenic regions of the chlamydial HSP-60 molecule which are recognized by antibody present in the sera of patients with urogenital tract infections of *C. trachomatis* who have developed or are at risk of developing postinfection sequelae.

MATERIALS AND METHODS

Bacterial strains. *C. trachomatis* L2/434/Bu has been previously described (26). Bacteriophage λ1059 and its host bacterial strain *Escherichia coli* Q359 (19) and the pUC plasmids and M13 phage systems and their respective *E. coli* host cell strains, TBI and XL-1 Blue, have been described previously (34). The expression plasmid pGEX (50) was the generous gift of G. B. Smith and G. Mitchell (Walter and Eliza Hall Institute).

Gene amplification. Template DNA for the polymerase chain reaction (PCR) was prepared from purified *C. trachomatis* EBs or from purified recombinant plasmid clones. EBs were lysed in 1% sodium dodecyl sulfate (SDS)–65 μg of proteinase K per ml, and the DNA was isolated following three phenol extractions, two chloroform extractions, and precipitation with ethanol. Oligonucleotides used as primers in the PCR were designed with 5' *Bam*HI and 3' *Eco*RI restriction endonuclease sites to allow directional cloning and were synthesized on an ABI 380B oligonucleotide synthesizer. PCR was performed following the manufacturer's instructions (Gene-Amp kit; Perkin-Elmer Cetus, Norwalk, Conn.). PCR products were purified prior to use by Centricon-30 filtration (Amicon Corp., Danvers, Mass.).

Selection and analysis of recombinant λ1059 phage. Construction of the *C. trachomatis* genomic library in λ1059 has been described previously (1). Recombinant phage was plated on *E. coli* Q359 by standard methods (31) at densities of approximately 10^3 PFU/150-mm (diameter) plate. Following overnight incubation to allow development of the plaques, the plates were sequentially overlaid twice with nitrocellulose disks (BA-85; Schleicher & Schuell, Keene, N.H.) and the disks were processed for DNA hybridization by standard methods (31). The nitrocellulose disks containing adsorbed phage DNA were probed with a chlamydial HSP-60-specific ^{32}P-end-labeled oligonucleotide, RS15. Derivation of this probe is described below (Results). Hybridization was conducted for 16 h at 42°C with a hybridization solution containing 1% SDS, 10% dextran sulfate, 50% formamide, 100 μg of denatured salmon sperm DNA per ml, and 1 M sodium chloride. Following hybridization, the membranes were washed twice in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at room temperature for a total of 30 min, twice in 2× SSC with 0.1% SDS at 49°C for a total of 60 min, and once in 0.1% SSC at room temperature

for 30 min. Plaques positive on both replicate disks were picked and reassayed at a density of ~100 PFU per plate, and isolated positive plaques were chosen and amplified. Four recombinant λ1059 clones were selected, and their DNA was isolated by standard procedures (31). The purified DNA was digested with *Bam*HI restriction endonuclease, and the specificity and size of the insert fragment in each clone were determined by Southern hybridization with the oligonucleotide probe RS15. The *Bam*HI-digested insert fragments were then cloned into pUC18.

DNA sequencing. Each of three λ1059 recombinant DNA inserts were cloned into pUC18, designated 2.1p12, 4.1p1, and 6.2p4, and mapped with restriction endonucleases and Southern hybridization by standard procedures (31). The enzymes used were *Bam*HI, *Eco*RI, *Hinc*II, *Hin*dIII, *Pst*I, and *Sac*I (Bethesda Research Laboratories, Gaithersburg, Md.; New England BioLabs, Inc., Beverly, Mass.). DNA restriction endonuclease fragments from the pUC clones or from PCR products were gel purified and cloned into M13mp18 or M13mp19. Sequencing of the M13 templates was by the dideoxy-chain termination method with *Taq* polymerase and fluorescence-labeled M13 primers (370 DNA sequencing system; Applied Biosystems Inc., Foster City, Calif.) and 370A automated DNA sequencer (Applied Biosystems).

Serological specificity. PCR-amplified regions of clone 2.1p12 were cloned into pGEX-2T with the *Bam*HI and *Eco*RI sites. Production and purification of glutathione S-transferase fusion proteins have been described previously (50). Protein concentrations were determined by the method of Lowry et al. (30), and purity was assessed by polyacrylamide gel electrophoresis and Coomassie blue staining. Polyacrylamide (12%)–N,N-methylenebisacrylamide (29:1) gel electrophoresis was performed by the method of Laemmli (27). Gels were transferred electrophoretically to nitrocellulose sheets (Schleicher & Schuell) as described by Towbin et al. (55). The fusion proteins were probed with rabbit serum specific to chlamydial Triton X-100-soluble HSP-60 (4; a generous gift of P. Bavoil, University of Rochester, Rochester, N.Y.) and with sera from patients with pelvic inflammatory disease and/or ectopic pregnancy. These patient sera have been described previously (60). Human sera were used at a dilution of 1:100, and immune reactions were identified with alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG), IgA, IgM (heavy plus light chains), 1:1,000 (Zymed Laboratories Inc., South San Francisco, Calif.). Prestained molecular weight standards were used: phosphorylase *b*, 110,000 Da; bovine serum albumin, 84,000 Da; ovalbumin, 47,000 Da; carbonic anhydrase, 33,000 Da; soybean trypsin inhibitor, 24,000 Da; and lysozyme, 16,000 Da (Bio-Rad).

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been submitted to the GenBank/EMBL data bank with accession number M58027.

RESULTS

Selection and analysis of λ1059 recombinant clones. Our interest in analyzing both the composition of chlamydial outer membrane protein antigens and their role in eliciting host immune responses prompted us to examine the composition of protein antigens of ~60,000 molecular weight. In addition to a 57,000- to 60,000-molecular-weight cysteine-rich outer membrane protein antigen (1), Bavoil et al. (5) characterized a different ~60,000-molecular-weight protein antigen that is soluble in solutions containing either Triton

X-100 or sarcosyl. Bavoil et al. (4) and Wagar et al. (60) have recently shown that the soluble protein is immunologically related to HSP-60. The highly conserved amino acid sequence characteristic of members of the HSP-60 family and the associated immunological cross-reactivity precluded the use of the antibody as a screening tool. Thus, we exploited the conservation of amino acid sequences in HSP-60s to design oligonucleotide primers for amplification of regions of the *C. trachomatis groEL* homolog by PCR (53). The amino acid sequences of HSP-60 analogs from *E. coli* (16), *Mycobacterium tuberculosis* (48), and *Coxiella burnetti* (59) were analyzed for regions of high homology that flanked nonconserved regions. The following four degenerate oligonucleotide primers, based on the reverse translation of these homologous regions and reflecting the codon bias of *C. trachomatis* (1), were synthesized: RS11 (5'-CTCGGAT CCGCNGTNAARGCNCCNGGNTTYGGNGAY-3'), RS12 (5'-CTCGGATCCGTNAARGTNACNYTNGGNCCNAA RGGN-3'), RS13 (5'-GTGGAATTCRTCNCCRAANCCN GGNGCYTTNACNGC-3'), and RS14 (5'-GTGGAATTC YTCYTGNARYTTYTCNCNRTCRTARTC-3'), where N = A, G, C, or T, R = A or G, and Y = C or T. The location and orientation of these primers relative to the amino acid residue comparison of the bacterial HSP-60 homologs is shown in Fig. 1A. These primers were used to amplify the nonconserved intervening regions from *C. trachomatis* genomic DNA. Two PCR products, 800 and 300 bp, were obtained and partially sequenced (53). The nucleic acid sequence from the 300-bp fragment revealed a DNA sequence that was unique to *C. trachomatis*. On the basis of the DNA sequence of this region, the following oligonucleotide, RS15, was synthesized: 5'-AAGCTTTAGAAGCT CGTTGCGAA-3'. Southern hybridization of *Bam*HI- and *Eco*RI-digested *E. coli* and *C. trachomatis* genomic DNAs probed with RS15 showed no binding of the oligonucleotide to the *E. coli* genomic DNA, whereas a band of ~450 bp was observed with *C. trachomatis* DNA (data not shown).

A genomic library of *C. trachomatis* in λ1059 was screened with the RS15 probe. Four positive recombinant λ1059 clones were selected, and the DNA was isolated and purified. Southern hybridization of purified DNA from the recombinant clones by using the RS15 probe showed that a common *Bam*HI restriction endonuclease fragment of ~4 kb was present in each λ clone (data not shown). This fragment from each λ1059 clone was purified and cloned into *Bam*HI-digested pUC18 plasmid. DNA was purified from pUC18 clones and mapped with restriction endonucleases. Figure 1B shows the restriction map for one clone, 2.1p12. All the recombinant pUC clones mapped identically, except for one clone, 4.3p1, that contained an additional 600-bp *Bam*HI fragment at the 3' end. Southern hybridization of restriction endonuclease digests of the pUC clones with RS15 localized the chlamydial HSP-60 gene to the 5' end of the insert DNA.

DNA sequence. Restriction endonuclease fragments derived from the pUC recombinants were cloned into M13mp18 and M13mp19 and sequenced as shown in Fig. 1C. The sequence determined confirmed that the clones contained the *C. trachomatis* homolog of the *groEL* gene family but were missing approximately 145 bp of the 5' end of the gene. We chose to isolate the portion of the HSP-60 gene missing from our recombinant clones by PCR extension from the known portion of the sequence. We reasoned that if *C. trachomatis* shared an HSP-60 gene organization similar to that seen in some other bacteria, such as *E. coli*, then the HSP-60 gene would exist 3' to a gene for a smaller protein (*groES*) in an operon, analogous to the *groE* operon of *E.*

coli. To extend the sequence, we constructed a degenerate oligonucleotide (5'-CCNGGATCCGANACNGTNGTNGC NGGNCCNGGNCGNTGG-3'; N = A, G, C, or T) on the basis of reverse translation of a consensus amino acid sequence derived from bacterial GroES sequences for use as the 5' primer in the PCR reaction. The 3' PCR primers were synthesized corresponding to two separate sequences complementary to the 5' end of the nucleotide sequence determined for the chlamydial *groEL* gene. We derived two separate PCR products by using the two different 3' primers (5'-CTCGAATTCTTTCTCAACGGTAACACCATCTTTA GTTAC-3' and 5'-AGCGAATTCTTTATGATGCTGAA CAGGCTTGCTGAT-3') for amplification. The two PCR products were cloned into the *Bam*HI and *Eco*RI sites of M13mp18 and M13mp19 and sequenced. Figure 1C shows the orientation of the two PCR products sequenced in relation to the recombinant plasmid clones and the *groE* operon of *E. coli*, which we used as a model of gene organization. Surprisingly, both PCR products were ~160 nucleotides longer than the expected products. However, the sequence of both products showed that the 3' end of the products agreed with the previous overlapping sequence determined from the recombinant clones. The degenerate 5' primer, reverse translated from the consensus sequences of GroES, fortuitously bound upstream of the initiation codon for an open reading frame encoding the GroES homolog.

Figure 2 shows the sequence compiled from both the PCR products and the restriction endonuclease fragments of the recombinant clones. There were two open reading frames. The first reading frame started with a methionine initiation codon and consisted of 306 nucleotides. Eight bases upstream of the initiation codon there was a sequence, GAG GAG, consistent with a Shine-Dalgarno complementarity (47). Downstream of this gene was a second, longer open reading frame consisting of 1,632 nucleotides, which also was preceded with a Shine-Dalgarno sequence, AAGGAG, 8 bp upstream of the open reading frame. Between the two open reading frames there was an intervening sequence of 39 nucleotides. Downstream of the larger open reading frame, there was an 11-nucleotide dyad followed by seven thymidines consistent with a rho-independent termination site (41). In those situations where the degeneracy of the code allows multiple codon choices for a given amino acid, codon usage reflected the bias of *C. trachomatis* for codons having A or T in the third nucleotide position (1).

During the preparation of the manuscript, Morrison et al. (35) published the sequence for the *groES* and *groEL* homologs of another *Chlamydia* species, *C. psittaci*. The gene organization of the *groES* and *groEL* homologs in both chlamydial species was similar. Comparison of the *groEL* and *groES* DNA sequences for each chlamydial species showed homologies of ~77 and ~81%, respectively. This compares to DNA homologies for the *C. trachomatis* sequences with *E. coli groES* and *groEL* of <50 and 60%, respectively. Although comparison of the aligned codons between the two *Chlamydia* species showed that approximately half of the codons from each gene contained at least one or more nucleotide substitutions, only 30 and 16% of these nonidentical codons resulted in an amino acid sequence difference between the two species for the *groES* and *groEL* homologs, respectively. The noncoding regions consisting of the 5'- and 3'-flanking sequences and the intergenic sequence showed extensive nucleotide sequence divergence between the *C. trachomatis* and *C. psittaci* operons. For example, the intergenic region of *C. trachomatis* contained 39 nucleotides, compared with 50 nucleotides comprising the

A. PROBE ISOLATION STRATEGY
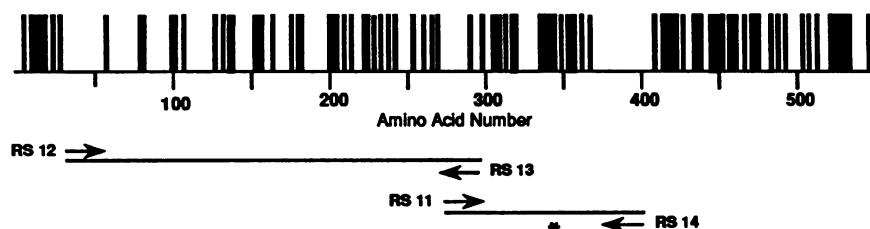
B. RESTRICTION MAP
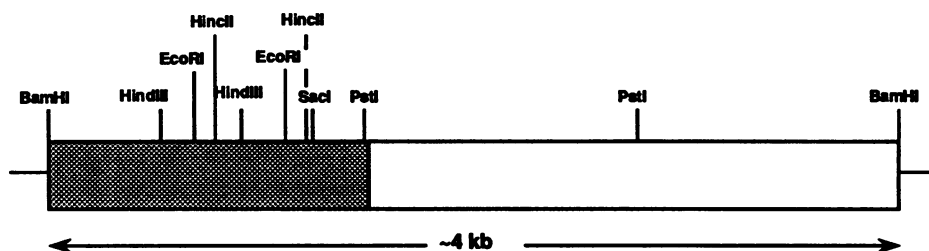
C. SEQUENCING STRATEGY
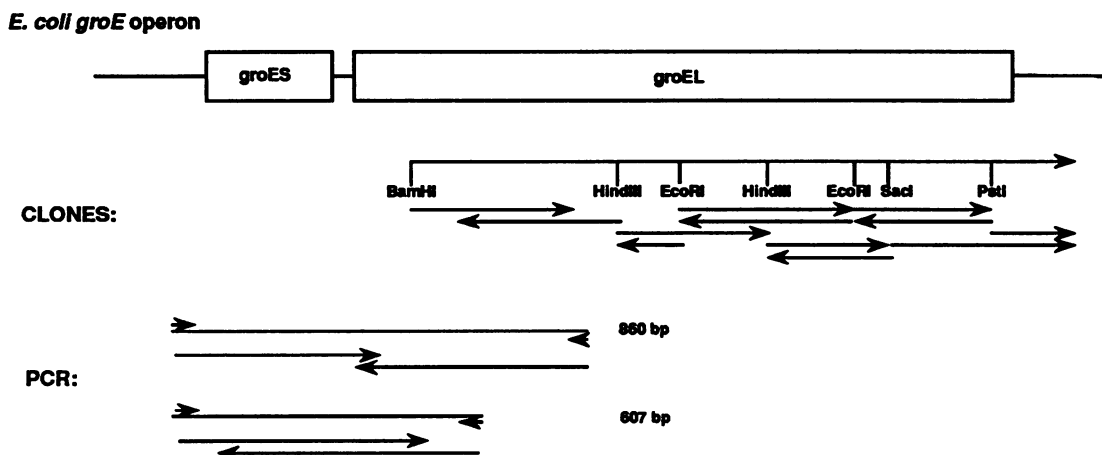
FIG. 1. (A) Isolation strategy for a *C. trachomatis*-specific DNA probe for the *groEL* homolog gene by using PCR. Vertical lines indicate amino acid differences between the GroEL homologs of *M. tuberculosis*, *C. burnetti*, and *E. coli*. Reverse transcription of regions with amino acid homology was used as a basis for the synthesis of degenerate oligonucleotide primers (RS11, RS12, RS13, and RS14). PCR amplication of the intervening regions produced two products for which a portion of each was sequenced (53). The location of a chlamydia-specific sequence used to define the synthetic nucleotide probe, RS15, is shown by the asterisk. (B) Restriction endonuclease map of the 4-kb *Bam*HI-*Bam*HI genomic fragment in pUC18 clone 2.1p12. The shaded area indicates the portion of the 4-kb fragment containing part of the *C. trachomatis groEL* (HSP-60) gene. (C) Sequencing strategy. The sequenced portion of the pUC18 clones and the sequenced PCR amplification products (arrowheads represent primers used for DNA amplification) are shown relative to the organization of the *E. coli groE* operon. Arrows show the fragments sequenced and the direction of sequencing.

C. psittaci intergenic region (35), with little homologous sequence alignment evident except for the Shine-Dalgarno sequences. These sequence differences reflect the lack of genomic DNA homology (<10%) between these species (12, 23) except in the coding regions of highly conserved genes, such as the *groES* and *groEL* genes.

Amino acid sequences. Figure 2 shows the translated amino acid sequences of both open reading frames. The smaller open reading frame, beginning with an initial methionine, encoded a protein consisting of 102 amino acids with a mass of 11,122 Da. The amino acid composition and characteristics of this *C. trachomatis* protein were similar to GroES of

```
                    G G C C T C A T A G A A T A T A A A A A T A C␣␣␣␣␣␣␣␣C T T A A A C
        1
       ATG TCA GAT CAA GCA ACG ACC CTC AAG ATT AAA CCT TTG GGA GAT AGA ATT TTA GTT AAA AGA GAA GAA GAA GCT
        M   S   D   Q   A   T   T   L   K   I   K   P   L   G   D   R   I   L   V   K   R   E   E   E   A
                                                                                                    50
       TCC ACT GCA AGA GGC GGA ATC ATT CTT CCT GAC ACT GCC AAG AAA AAG CAA GAT AGA GCT GAA GTT GTA GCT CTA
        S   T   A   R   G   G   I   I   L   P   D   T   A   K   K   K   Q   D   R   A   E   V   V   A   L

       GGA ACA GGC AAA AAA GAT GAT AAA GGG CAG CAA CTT CCT TTT GTA GTT CAG GTT GGT GAC ATC GTT TTA ATT GAT
        G   T   G   K   K   D   D   K   G   Q   Q   L   P   F   V   V   Q   V   G   D   I   V   L   I   D
                                                                                                    100
       AAA TAT TCT GGC CAA GAA CTC ACT GTC GAA GGT GAA GAG TAC GTC ATC GTT CAA ATG AGC GAA GTT ATC GCA GTT
        K   Y   S   G   Q   E   L   T   V   E   G   E   E   Y   V   I   V   Q   M   S   E   V   I   A   V
           102                                                                  1
       CTG CAA TAA  A A C T A A G A G A G T G A A G T A A G A T T T␣␣␣␣␣␣␣C G C A T C A  ATG GTC GCT
        L   Q   STOP                                                                     M   V   A

       AAA AAC ATT AAA TAC AAC GAA GAA GCC AGA AAG AAA ATT CAA AAA GGA GTT AAG ACT TTA GCT GAA GCT GTA AAA
        K   N   I   K   Y   N   E   E   A   R   K   K   I   Q   K   G   V   K   T   L   A   E   A   V   K
                                                    50
       GTC ACT CTA GGG CCT AAA GGA CGA CAT GTT GTC ATA GAT AAA AGC TTC GGA TCC CCT CAA GTA ACT AAA GAT GGT
        V   T   L   G   P   K   G   R   H   V   V   I   D   K   S   F   G   S   P   Q   V   T   K   D   G

       GTT ACC GTT GCG AAA GAA GTT GAG CTT GCC GAC AAA CAT GAA AAT ATG GGC GCT CAA ATG GTC AAA GAA GTC GCC
        V   T   V   A   K   E   V   E   L   A   D   K   H   E   N   M   G   A   Q   M   V   K   E   V   A
                                                                                        100
       AGC AAA ACT GCT GAC AAA GCT GGA GAC GGA ACT ACA ACA GCT ACT GTT CTT GCT GAA GCT ATC TAT ACA GAA GGA
        S   K   T   A   D   K   A   G   D   G   T   T   T   A   T   V   L   A   E   A   I   Y   T   E   G

       TTA CGC AAT GTA ACA GCT GGA GCA AAT CCA ATG GAC CTC AAA CGA GGT ATT GAT AAA GCT GCT AAG GTT GTT GTT
        L   R   N   V   T   A   G   A   N   P   M   D   L   K   R   G   I   D   K   A   A   K   V   V   V
                                                                                    150
       GAT CAA GTC AAA AAA ATC AGC AAG CCT GTT CAG CAT CAT AAA GAA ATT GCT CAA GTT GCA ACA ATT TCT GCT AAT
        D   Q   V   K   K   I   S   K   P   V   Q   H   H   K   E   I   A   Q   V   A   T   I   S   A   N

       AAT GAT GCA GAA ATC GGG AAT CTG ATT GCT GAA GCA ATG GAG AAA GTT GGT AAA AAC GGC TCT ATC ACT GTT GAA
        N   D   A   E   I   G   N   L   I   A   E   A   M   E   K   V   G   K   N   G   S   I   T   V   E
                                                                          200
       GAA GCA AAA GGA TTT GAA ACC GTT TTG GAT GTT GTT GAC GGA ATG AAT TTC AAT AGA GGT TAC CTC TCT AGC TAC
        E   A   K   G   F   E   T   V   L   D   V   V   D   G   M   N   F   N   R   G   Y   L   S   S   Y

       TTC GCA ACA AAT CCA GAA ACT CAA GAA TGT GTA TTA GAA GAA GCT TTG GTT CTA ATC TAC GAT AAG AAA ATA TCT
        F   A   T   N   P   E   T   Q   E   C   V   L   E   E   A   L   V   L   I   Y   D   K   K   I   S
                                                                  250
       GGG ATC AAA GAT TTC CTT CCT GTT TTA CAA CAA GTT GCT GAA TCC GGC CGT CCT CTT CTT ATT ATA GCA GAA GAC
        G   I   K   D   F   L   P   V   L   Q   Q   V   A   E   S   G   R   P   L   L   I   I   A   E   D

       ATT GTA GGC GAA GCG TTA GCT ACT TTG GTC GTG AAC AGA ATT CGT GGA GGA TTC CGG GTT TGC GCA GTT AAA GCT
        I   V   G   E   A   L   A   T   L   V   G   N   R   I   R   G   G   F   R   V   C   A   V   K   A
                                                                              300
       CCA GGC TTT GGA GAT AGA AGA AAA GCT ATG TTT GAA GAC ATC GCT ATC TTA ACT GGC GGT CAA CTC ATT AGC GAA
        P   G   F   G   D   R   R   K   A   M   F   E   D   I   A   I   L   T   G   G   Q   L   I   S   E

       GAG TTG GGC ATG AAA TTA GAA AAC GCT AAC CTA GCT ATG TTA GGT AAA GCT AAA AAA GTT ATC GTT TCT AAA GAA
        E   L   G   M   K   L   E   N   A   N   L   A   M   L   G   K   A   K   K   V   I   V   S   K   E
                                                                                  350
       GAC ACG ACC ATC GTC GAA GGA ATG GGT GAA AAA GAA GCT TTA GAA GCT CGT TGC GAA AGC ATC AAA AAA CAA ATT
        D   T   T   I   V   E   G   M   G   E   K   E   A   L   E   A   R   C   E   S   I   K   K   Q   I

       GAA GAC AGC TCT TCT GAT TAC GAT AAA GAA AAA CTC CAA GAG CGT CTT GCT AAG CTC TCT GGT GGA GTA GCA GTC
        E   D   S   S   S   D   Y   D   K   E   K   L   Q   E   R   L   A   K   L   S   G   G   V   A   V
                                                                      400
       ATT CGC GTT GGA GCT GCA ACA GAG ATT GAG ATG AAA GAG AAA AAA GAT CGT GTA GAC GAT GCT CAA CAT GCT ACA
        I   R   V   G   A   A   T   E   I   E   M   K   E   K   K   D   R   V   D   D   A   Q   H   A   T

       ATC GCT GCT GTT GAA GAA GGA ATT CTT CCT GGT GGA GGA ACA GCA TTA ATC CGT TGT ATC CCT ACT CTT GAA GCC
        I   A   A   V   E   E   G   I   L   P   G   G   G   T   A   L   I   R   C   I   P   T   L   E   A
                                                                                  450
       TTC TTG CCA ATG TTG ACT AAT GAA GAT GAG CAA ATT GGA GCT CGC ATT GTT TTG AAA GCT CTT TCC GCT CCT TTG
        F   L   P   M   L   T   N   E   D   E   Q   I   G   A   R   I   V   L   K   A   L   S   A   P   L

       AAA CAA ATT GCT GCA AAC GCA GGA AAA GAA GGT GCT ATC ATC TTC CAA CAA GTT ATG TCC CGT TCT GCG AAC GAA
        K   Q   I   A   A   N   A   G   K   E   G   A   I   I   F   Q   Q   V   M   S   R   S   A   N   E
                                                                              500
       GGA TAT GAT GCA TTG CGT GAT GCA TAC ACA GAT ATG CTT GAA GCT GGT ATT TTA GAT CCT GCT AAA GTA ACC CGT
        G   Y   D   A   L   R   D   A   Y   T   D   M   L   E   A   G   I   L   D   P   A   K   V   T   R

       TCT GCT TTA GAA AGC GCG GCT TCC GTA GCT GGA TTA CTT TTG ACA ACA GAA GCT CTC ATT GCA GAG ATT CCA GAA
        S   A   L   E   S   A   A   S   V   A   G   L   L   L   T   T   E   A   L   I   A   E   I   P   E
                                          544
       GAA AAA CCT GCT GCA GCT CCA GCA ATG CCT GGC GCA GGA ATG GAC TAT TAA  T T C C T C T A A T G G G A A C
        E   K   P   A   A   A   P   A   M   P   G   A   G   M   D   Y   STOP
                                                                     ──────────  ◄──────
        A A A T A G A T T C T T C G A G C C T C G T T T C C C A A A A G G A A C G A G G C T T T T T T T T A
        G A T T C C T A A T A T T T C T C T A T T C C T C T A T C G T A A A C A T C T A G T G C T T A C G A
        C C A T C C T T T T C T A T G T T T A A A C T A A T C A A G A G C G A T T T C T C A T A G C C T G T
```
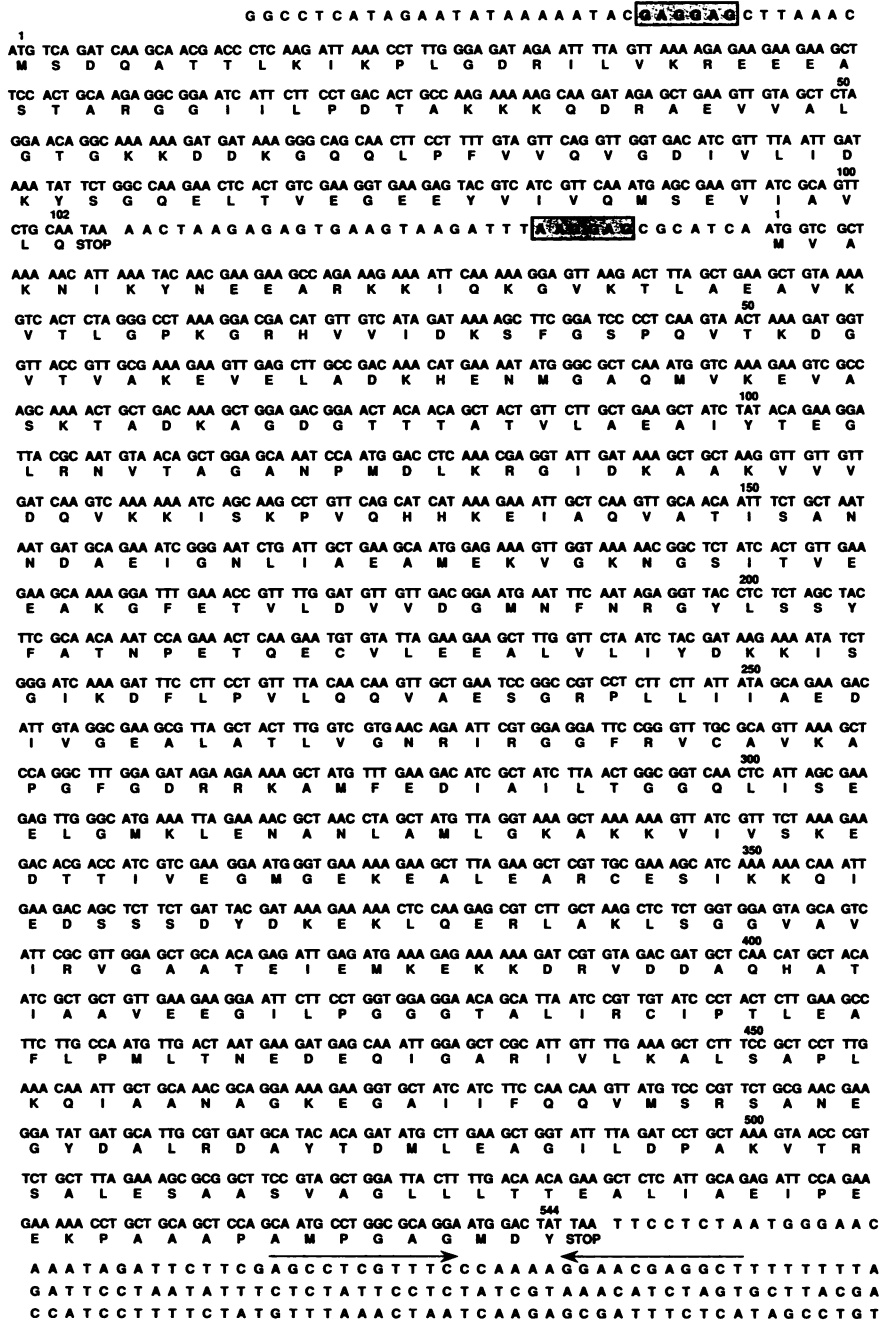
FIG. 2. Nucleotide sequence of the *C. trachomatis groE* operon. The amino acid sequences translated from the *groES* and *groEL* genes are indicated below the nucleotide sequence. The ribosome-binding sites present in the 5'-flanking and intergenic sequences are shown by shaded boxes, and the 11-bp dyad symmetery of the proposed transcription termination site present in the 3'-flanking sequence is shown with arrows. Numbers above each of the translated amino acid sequences refer to amino acid positions in each protein.

E. coli (16), *C. burnetti* (59), *M. tuberculosis* (3), and *C. psittaci* (35). The amino acid sequences of each of the GroES analogs were aligned with each other and the *C. trachomatis* sequence (Fig. 3). Amino acid sequence homology was highest between the GroES homologs of the two chlamydial species (Table 1), while homology of the *C. trachomatis* sequence with the other bacterial GroES analogs was less than 50%. Areas of amino acid conservation and diversity were dispersed throughout the protein. However, one notable difference between the two chlamydial amino acid sequences and the other bacterial analogs was the insertion of up to seven additional amino acid residues at the amino terminus of the chlamydial homologs compared with the other bacterial sequences.

The larger open reading frame encoded, beginning with a methionine, a 57,956-Da protein consisting of 544 amino acids. This sequence was homologous to the composition of other bacterial GroEL sequences, as well as to the plant
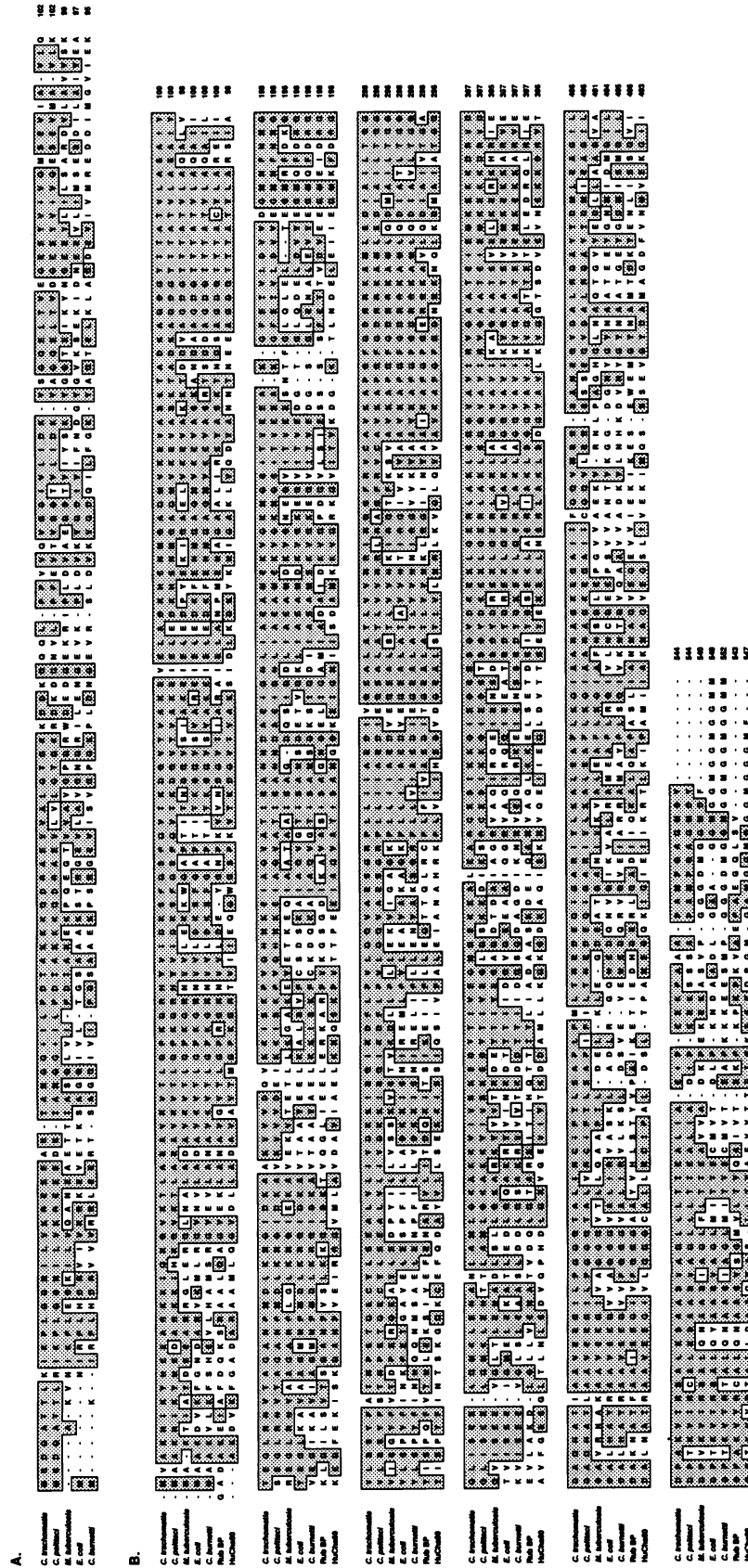
FIG. 3. (A) Comparison of the deduced amino acid sequence of the *C. trachomatis* GroES homolog with the GroES counterparts of *C. psittaci* (35), *M. tuberculosis* (3), *E. coli* (16), and *C. burnetii* (58). (B) Comparison of the translated amino acid sequence of *C. trachomatis* GroEL with GroEL proteins of *C. psittaci* (35), *M. tuberculosis* (48), *E. coli* (16), *C. burnetii* (59), plant Rubisco-binding protein from *Triticum aestivium* (16), and the mature form of human mitochondrial chaporonin 60 protein (18, 61). Shaded boxed areas indicate regions of amino sequence homology with *C. trachomatis* sequence. Gaps introduced to facilitate sequence alignment are indicated by hyphens.

TABLE 1. Percent amino acid homology of *C. trachomatis* proteins with GroES and GroEL analogs

Species or protein (reference)	Percent homology with *C. trachomatis*	
	GroES	GroEL
Chlamidia psittaci (35)	85	93
Mycobacterium tuberculosis (3, 48)	44	57
Escherichia coli (16)	31	60
Coxiella burnetti (59)	33	61
Rub BP (16)		48
HuCha60 (18, 61)		48

Rubisco-binding protein (16) and the human mitochondrial chaperonin 60 proteins (18, 61) (Fig. 3). The *C. trachomatis* sequence shared extensive amino acid homology with the *C. psittaci* HSP-60 sequence (~93%); however, 37 of 544 amino acids differed (Table 1). Homology to the HSP-60s of the other bacterial strains was ~60%, and with the plant and human homologs it was 48%. There were extensive areas of amino acid sequence which were conserved throughout all seven analogs. However, there were several regions where the *Chlamydia* HSP-60 showed divergence from the other HSP-60s, most notably the amino terminus (Asn-5 → Gln-18), the carboxyl terminus (Glu-520 → Thr-544), and interior regions (Ala-124 → Glu-143, Ala-205 → Arg-245, Ala-320 → Ser-356, Ile-420 → Ile-440, and Gly-464 → Ala-493). Given the cysteine-rich nature of chlamydial outer membrane complexes, it is significant that examination of both the number and position of cysteine residues in the various GroEL sequences revealed that *C. trachomatis* and *C. psittaci* share four conserved cysteine residues in regions of the molecule where they have not been highly conserved in the other analogs (Fig. 4).

Following submission of the manuscript, Morrison et al. published the nucleic acid sequence for the *groE* operon and the translated amino acid sequences for the HSP-60 (GroEL) and GroES homologs of the trachoma biovar A of *C. trachomatis* (37). Comparison of the HSP-60 amino acid sequences from the trachoma biovar A with the translated amino acid described herein for the lymphogranuloma venereum (LGV) biovar L2 revealed six amino acid residues which differed between the HSP-60s of the two biovars.

These were as follows: Ala-124 (L2) versus Val-124 (A), Val-131 (L2) versus Ile-131 (A), Asp-191 (L2) versus Glu-191 (A), Glu-217 (L2) versus Asp-217 (A), Val-236 (L2) versus Ile-236 (A), and Val-255 (L2) versus Glu-255 (A). Comparison of the translated amino acid sequences for the GroES homologs from the trachoma biovar A (37) and the LGV biovar L2 showed that two amino acid residues differed: Val-48 (L2) versus Leu-48 (A) and Val-65 (L2) versus Glu-65 (A).

Antigenic regions of *C. trachomatis* HSP-60. Wagar et al. (60) demonstrated the presence of antibodies against *C. trachomatis* HSP-60 in 81% of ectopic pregnancy patients with evidence of chlamydial infection. To unequivocally demonstrate that the Triton X-100-soluble antigen detected by Wagar et al. (60) and Bavoil et al. (4) was HSP-60 and to define the antigenic regions of this molecule, we produced a series of five glutathione S-transferase fusion peptides for analysis by immunoblotting. The immunoblots were probed with representative patient sera derived from the same patient pool as used in the study by Wagar et al. (60). These sera were selected from pelvic inflammatory disease and ectopic pregnancy patients possessing *C. trachomatis* micro-immunofluorescence titers ≥1:512 and were defined as reactive (patient sera 1 to 5) or nonreactive (patient sera 6 to 8) to HSP-60. Figure 5A demonstrates that all of the patient sera were reactive against either or both of the major *C. trachomatis* surface antigens, the major outer membrane protein and the 60-kDa cysteine-rich protein. Figure 5B shows the delineation of the patient sera as HSP-60 reactive or nonreactive to the Triton X-100-soluble chlamydial HSP-60. Rabbit monospecific sera against major outer membrane protein, 60-kDa cysteine-rich protein, and chlamydial HSP-60 served as control sera delineating the migration position of their respective antigens on the immunoblots, as well as confirming the differential separation of the two 60,000-Da antigens from the outer membrane complex and Triton X-100-soluble fractions of EBs (60).

The glutathione S-transferase fusion proteins contained portions of the *C. trachomatis* HSP-60, delineating the following regions of the molecule: Met-1 → Lys-51, Thr-50 → Glu-143, Thr-50 → Arg-266, Ala-274 → Ala-402, and Ala-405 → Tyr-544. Because all of the fusion proteins contained glutathione S-transferase as a portion of the expressed proteins, the patient sera were tested for reactivity
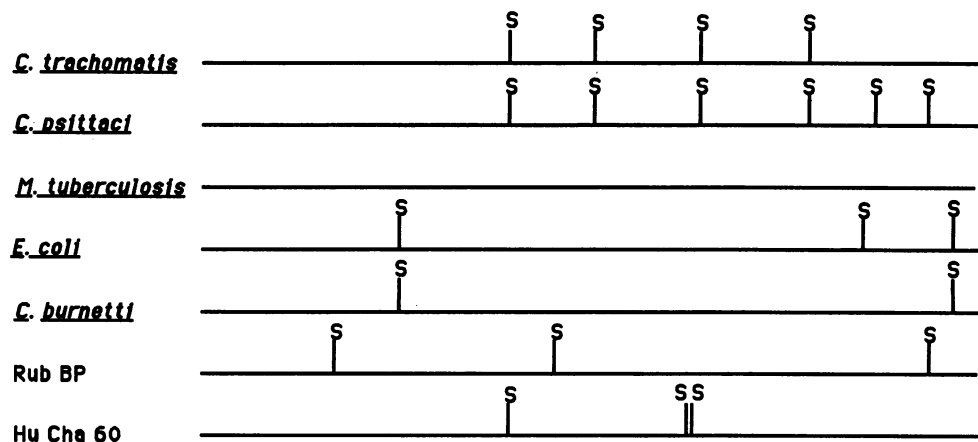

FIG. 4. Comparison of the linear distribution of cysteine residues in the amino acid sequences of GroEL homologs. Each vertical line indicates the location of a single cysteine residue in the GroEL homologs of *C. trachomatis*, *C. psittaci* (35), *M. tuberculosis* (48), *E. coli* (16), *C. burnetti* (59), plant Rubisco binding protein (16), and human mitochondrial chaperonin 60 protein (18, 61).
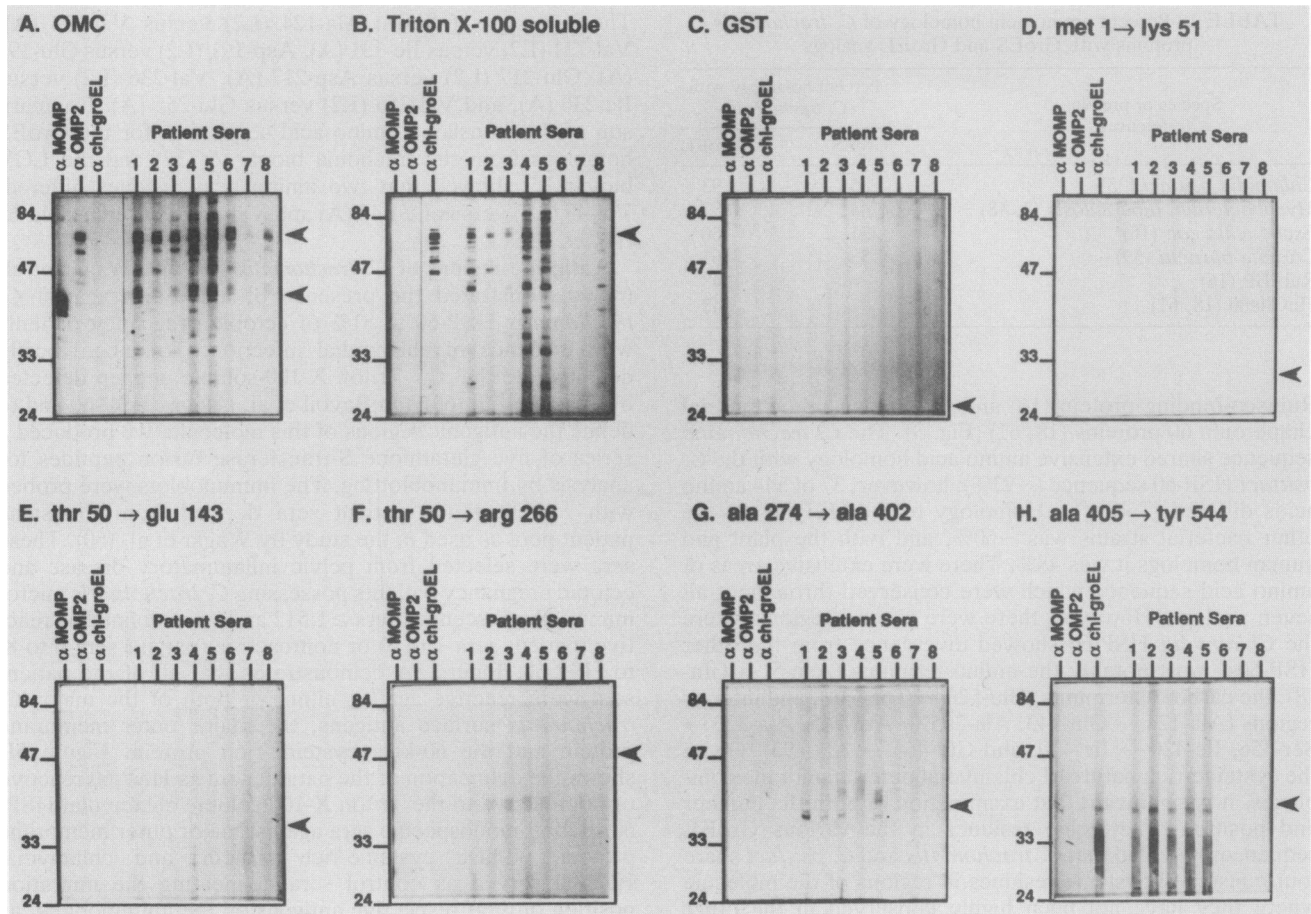
FIG. 5. Immunoblot analysis of the antigenic regions of *C. trachomatis* HSP-60. The outer membrane complex (OMC) antigen fraction and the Triton X-100-soluble antigen fraction of *C. trachomatis* EBs, the glutathione S-transferase (GST) control protein, and the glutathione S-transferase fusion proteins containing regions of the *C. trachomatis* HSP-60 were subjected to 10% polyacrylamide gel electrophoresis under denaturing and reducing conditions and were electrotransferred to nitrocellulose. Immunoblots were probed with rabbit antisera specific for major outer membrane protein (lanes α MOMP), the 60-kDa cysteine-rich outer membrane protein (α OMP2), and chlamydial HSP-60 (α chl-groEL), or with patient sera from individuals with chlamydial microimmunofluorescence titers ≥512 (lanes 1 to 8) against OMC (A), Triton X-100-soluble fraction (B), GST (molecular weight [MW], ~26,000) (C), fusion peptide Met-1 → Lys-51 (MW, ~32,000) (D), fusion peptide Thr-50 → Glu-143 (MW, ~36,000) (E), fusion peptide Thr-50 → Arg-266 (MW, ~50,000) (F), fusion peptide Ala-274 → Ala-402 (MW, ~40,000) (G), and fusion peptide Ala-405 → Tyr-544 (MW, ~40,000) (H). Arrowheads indicate electrophoretic migration positions for fusion proteins based on estimated MWs. Numbers to the left of each panel refer to MWs (thousands).

to glutathione S-transferase. None of the patient sera reacted with glutathione S-transferase (Fig. 5C). Figures 5D to H show the reactivity of the patient sera with the fusion proteins defining different regions of the HSP-60. Sera from infected patients not exhibiting reactivity to chlamydial HSP-60 in the Triton X-100-soluble EB fraction (sera 6 to 8) did not detectably react with any of the fusion proteins. Sera from uninfected pelvic inflammatory disease or ectopic pregnancy patients were uniformly nonreactive on the immunoblots (data not shown). Sera from *C. trachomatis*-infected patients which were reactive to HSP-60 (sera 1 to 5) recognized fusion proteins containing Ala-274 → Ala-402 (Fig. 5G) and Ala-405 → Tyr-544 (Fig. 5H), representing the carboxyl-terminal half of the HSP-60 molecule. Sera from all five of the HSP-60-reactive patients recognized the Ala-405 → Tyr-544 sequence that encompasses the carboxyl terminus, whereas only four of the five recognized the region Ala-274 → Ala-402. Reactivity against the other three fusion proteins (Fig. 5D to F) was negligible. These data provided

molecular confirmation of the conclusions of Wagar et al. (60) and Bavoil et al. (4) and furthermore showed that the antibody reactivity present in human sera against HSP-60 is directed toward the carboxyl-terminal end of the *C. trachomatis* HSP-60 molecule.

DISCUSSION

Heat shock proteins or stress proteins are a class of highly conserved proteins that have important functions in cellular metabolism and aid cells in dealing with adverse environmental stimuli. There are several families of heat shock proteins classified by the approximate molecular weight of their constituents (for a review, see reference 29). Members of the HSP-60 family are found in both procaryotes and eucaryotes, but in eucaryotes their presence appears restricted to the mitochondria and chloroplast organelles. HSP-60 homologs that are antigenically cross-reactive are serologically detectable in such a wide range of different

gram-negative bacteria that they have been referred to as common antigen (51, 52). Triton X-100-solubilized proteins of ~57,000 molecular weight from *C. trachomatis* (4) and *C. psittaci* (35) EBs have been demonstrated to be antigenically related to similar molecular weight proteins found in numerous gram-negative bacteria. This protein can be differentially purified and separated from another similar molecular weight immunodominant *C. trachomatis* protein (5). The gene encoding a *C. psittaci* 57,000-molecular-weight protein cross-reactive with bacterial common antigen has recently been isolated and cloned (33, 35).

We used the high degree of structural conservation characteristic of other sequenced HSP-60 proteins and the technique of PCR amplification to product a DNA probe specific for the *C. trachomatis* HSP-60 homolog (53). This probe allowed us to isolate recombinant clones from a genomic library of *C. trachomatis* and to sequence the gene for the chlamydial HSP-60 (*chl-groEL*) as well as a second smaller gene encoding the chlamydial GroES. These genes were homologs of *groEL* and *groES* by the following criteria: (i) the high degree of amino acid sequence homology with other GroES and GroEL analogs, (ii) the overall similarity of amino acid composition and protein characteristics among HSP-60s, (iii) the identical organization of the *C. trachomatis groES* and *groEL* genes with those of the *E. coli groE* operon, and (iv) antigenic cross-reactivity.

The organization of the *C. trachomatis* genes was identical to the organization of the *groES* and *groEL* genes of a second chlamydial species, *C. psittaci* (35). The *groE* operon structure is not, however, a universal organization in which the genes for both proteins are controlled by a single set of heat shock promoters. The homologs of *Mycobacterium* species do not show the linkage of the *groES* and *groEL* genes in a single operon (49). Furthermore, although *groEL* homologs have been found and sequenced in eucaryotes, no *groES* homologs have been found (46). Pulse-label experiments with chlamydia demonstrated both the increased expression of some proteins and the new expression of 10 to 20 other proteins following a shift to elevated culture temperatures (42). One of the increased expression products was identified as the chlamydial groEL homolog. The induction of the heat shock response was at the level of transcription, and thus as in other systems the chlamydial *groEL* operon is transcriptionally active in response to heat shock. In addition, in *C. trachomatis*, large amounts of the protein from this operon are produced constitutively (42).

The high degree of structural and amino acid sequence conservation among HSP-60s is reflective of their critical role in cellular metabolism under both stressful and normal growth conditions. Although all the functions and the mechanism of action for GroEL and GroES proteins are not completely understood, it appears that the GroEL protein forms a homooligomer composed of two stacked rings of 7 subunits each that associate with a ring structure of 6 to 8 GroES subunits to form an operational complex (46). These complexes are important for the assembly and disassembly of multimeric subunit protein complexes (14, 44). Evidence also suggests that the GroEL-GroES complex functions in aiding proteins to fold correctly and assume the proper structural conformation during synthesis (6) and in the maintenance of protein conformations necessary for intracellular transport (9).

Chlamydiae have a unique developmental cycle involving the conversion between two distinct developmental forms. The extracellular EB form possesses an outer membrane composed of three cysteine-rich proteins that form complex,

disulfide cross-linked oligomers and provide a rigid and impermeable surface structure. Conversion to the intracellular replicative reticulate body form, possessing a flexible and permeable outer membrane, requires the structural rearrangement of the membrane, including the dissociation of the disulfide linkages (39). It is attractive to speculate that the chlamydial GroEL and GroES analogs participate in mediating the unique oligomeric outer membrane rearrangement process. The apparent cellular location of the chlamydial HSP-60 is consistent with this hypothesis. In other bacteria, HSP-60 is normally located in the cytoplasm (46); however, in chlamydiae large amounts of HSP-60 can be obtained merely by washing the organisms with isotonic solutions (4). This observation suggests that much of the chlamydial HSP-60 is associated in the interstices of the outer membrane. Bavoil et al. (4) demonstrated with thiopropyl affinity chromatography that the *C. trachomatis* HSP-60 has reactive sulfhydryls. It was further found that two different forms of the HSP-60 protein exist in *C. trachomatis* EBs. The predominant form is reduced and peripherally membrane associated, and a lesser amount is oxidized and bound by disulfide linkages to the outer membrane. Examination of both the number and positions of cysteine residues in the various GroEL sequences revealed that *C. trachomatis* and *C. psittaci* shared four conserved cysteine residues in a region of the molecule in which they have not been highly conserved in the other analogs. These results suggest that the chlamydial GroES-GroEL complex is involved in the assembly and disassembly of the EB outer membrane complex and/or the maintenance of major outer membrane protein in its monomeric dissociated state in the reticulate body.

The immunodominance of heat shock proteins from the HSP-60 and HSP-70 families as antigens eliciting antibody responses in a wide range of infections by helminths, protozoa, and bacteria is well documented (20). Immune responses specific for the HSP-60 family have been best studied for the *Mycobacterium tuberculosis* HSP-60 (for a review, see reference 64). These studies demonstrate that HSP-60 is the immunodominant antigen for both cellular and antibody immune responses. Of 24 monoclonal antibodies generated in mice against *Mycobacterium leprae*, 7 were directed against *M. leprae* HSP-60 (63). In mice immunized with *M. tuberculosis*, limiting dilution analysis estimated that 20% of the T cells that responded to *M. tuberculosis* were specific for HSP-60 (21). By using synthetic peptides to define immunoreactive epitopes, Lamb et al. (28) and Munk et al. (38) demonstrated T-cell responses against epitopes shared by mycobacterial and human HSP-60 homologs. Murine cytolytic T cells generated against *Mycobacterium* HSP-60 lysed target cells consisting of not only macrophages pulsed with HSP-60 peptides but also macrophages stressed by a variety of stimuli (24). The shared homology between bacterial and human HSP-60 cognates provides a basis for the generation of autoimmune responses. Additionally, the demonstration of cross-reactivity between a T-cell-recognized epitope of *M. leprae* and a similar peptide sequence present on HLA-DR2 major histocompatibility antigens may also provide a basis for development of autoreactive responses (2).

Genitourinary infection with *C. trachomatis* results in an increased incidence of tubal infertility and ectopic pregnancy in women. Brunham et al. (7, 8) studied the serological immune response following sexually transmitted chlamydial infections in patients with tubal infertility and in patients developing postabortal salpingitis. The tubal infertility study

found that the majority of infertile patients in the study had antibody recognizing a 57,000-molecular-weight antigen, whereas only 1 of 11 chlamydial seropositive fertile women had serum antibody against this protein. Reactivity against this antigen, therefore, correlated with a negative outcome following infection. The study of postabortal salpingitis patients, however, suggested a protective role for antibody against this antigen. Unfortunately, the identity of the antigen in both studies was unknown and could have been the immunodominant 57,000- to 60,000-molecular-weight cysteine-rich protein (40). Wagar et al. (60) demonstrated that 31% of pelvic inflammatory disease patients and 81% of ectopic pregnancy patients with high antichlamydial antibody titers had antibody against the Triton X-100-extractable ~60,000-molecular-weight protein, and this protein was serologically cross-reactive with mycobacterial and *E. coli* HSP-60s. Thus, unlike mycobacterially and schistosomally elicited serological responses to HSP-60, serological responses to chlamydial HSP-60 are often not observed. Our current study extends these observations, demonstrates reactivity in these same patients to sequence-defined chlamydial HSP-60 peptides, and suggests that the reactive epitopes are contained within the carboxyl-terminal half of the molecule. The last observation is reminiscent of the serological responses to *M. tuberculosis* and *M. leprae* HSP-60 in which species-specific and the majority of immunodominant epitopes are located within the carboxyl-terminal region of the molecule (63).

Genitourinary infections of males with *C. trachomatis* are associated with the development of a postinfection sequela, Reiter's syndrome, a form of sexually acquired, reactive arthritis. Epidemiologic studies have estimated the incidence of acute *C. trachomatis* infection immediately preceding the onset of sexually acquired arthritis as 42 to 69% of cases (13, 22, 25, 58), and chlamydial antigens have been demonstrated in a patient with rheumatoid arthritis (10). Although the identity of the chlamydial constituents associated with the development of reactive arthritis in these cases is not known, reactivity against the mycobacterial HSP-60 homolog has been demonstrated in patients with rheumatoid arthritis (11, 45, 56). Adjuvant-induced arthritis in rats has been used as an experimental model of rheumatoid arthritis. In this model, arthritogenic T cells from the rats recognize a nonapeptide epitope on *M. tuberculosis* HSP-60 which is cross-reactive with a self antigen of joint cartilage (57). Recent evidence indicates that a subset of T cells, γδ T cells, react with antigens on autologous cells in which heat shock responses and heat shock antigens are induced (43), and γδ T cells isolated from the synovial fluid of a patient with rheumatoid arthritis recognized mycobacterial HSP-60 (17). The association of γδ-phenotype T cells with mucosal epithelial tissues (20) and the mucosal site of *C. trachomatis* infection raises provocative questions on the role of both this T-cell subset and other T-cell subsets with specificity directed toward HSP-60 in mediating either protective or immunopathology-inducing responses during infection.

The role that HSP-60 plays in the immune response to bacterial infections is incompletely understood. The above studies demonstrate a correlation of immunoreactivity against HSP-60s and immunopathology. However, anti-HSP-60 reactivity is demonstrable in normal individuals with no evidence of clinical disease (38) and in some cases may be interpreted as protective against bacterial infection (64). Definition of the recognized epitopes on HSP-60s for a variety of pathogenic bacteria and examination of recognized epitopes within the variety of immunopathogenic

conditions caused by a single bacterium such as *C. trachomatis* may facilitate interpretation of the complex immunoregulatory process separating protective and pathology-inducing immune responses.

REFERENCES

1. **Allen, J. E., and R. S. Stephens.** 1989. Identification by sequence analysis of two-site posttranslational processing of the cysteine-rich outer membrane protein 2 of *Chlamydia trachomatis* serovar L2. J. Bacteriol. **171:**285–291.
2. **Anderson, D. C., W. C. A. van Schooten, M. E. Barry, A. A. M. Janson, T. M. Buchanan, and R. R. P. de Vries.** 1988. A *Mycobacterium leprae*-specific human T cell epitope cross-reactive with an HLA-DR2 peptide. Science **242:**259–261.
3. **Baird, P. N., L. M. C. Hall, and A. R. M. Coates.** 1988. A major antigen from *Mycobacterium tuberculosis* which is homologous to the heat shock proteins groES from *E. coli* and the htpA gene product of *Coxiella burneti*. Nucleic Acids Res. **16:**9047.
4. **Bavoil, P., R. S. Stephens, and S. Falkow.** 1990. A soluble 60,000 M.W. antigen of Chlamydia spp. is a homolog of Escherichia coli groEL. Mol. Microbiol. **4:**461–469.
5. **Bavoil, P., L. Palmer, S. Falkow, and D. Gump.** 1986. Characterization and cloning of cysteine-rich proteins from *Chlamydia trachomatis*: a preliminary report, p. 97–100. *In* D. Oriel, G. Ridgway, J. Schachter, D. Taylor-Robinson, and M. Ward (ed.), Chlamydial infections. Cambridge University Press, Cambridge.
6. **Bochkareva, E. S., N. M. Lissin, and A. S. Girshovich.** 1988. Transient association of newly synthesized unfolded proteins with the heat-shock GroEL protein. Nature (London) **336:**254–257.
7. **Brunham, R. C., B. Binns, J. McDowell, and M. Paraskevas.** 1986. *Chlamydia trachomatis* infection in women with ectopic pregnancy. Obstet. Gynecol. **67:**772–776.
8. **Brunham, R. C., L. P. Maclean, B. Binns, and R. W. Peeling.** 1985. *Chlamydia trachomatis*: its role in tubal infertility. J. Infect Dis. **152:**1275–1282.
9. **Cheng, M. Y., F. U. Hartl, J. Martin, R. A. Pollock, F. Kalousek, W. Neupert, E. M. Hallberg, R. L. Hallberg, and A. L. Horwich.** 1989. Mitochondrial heat-shock protein HSP-60 is essential for assembly of proteins imported into yeast mitochondria. Nature (London) **337:**620–625.
10. **Ford, D. K., G. D. Reid, S. Magge, and H. R. Schumacher.** 1988. Synovial lymphocyte response to chlamydia stimulation associated with intrasynovial chlamydial antigen in a patient with "rheumatoid arthritis." Arthritis Rheum. **31:**914–917.
11. **Gaston, J. S. H., P. F. Life, L. Bailey, and P. A. Bacon.** 1988. Synovial fluid T cells and 65 kD heat-shock protein. Lancet **ii:**856.
12. **Gerloff, R. K., D. B. Ritter, and W. O. Watson.** 1970. Studies on thermal denaturation of DNA from various chlamydiae. J. Infect. Dis. **121:**65–69.
13. **Ghinsberg, R. C., D. Zigner, R. Shoshani-Perimutter, M. Elyan, and Y. Nitzan.** 1989. *Chlamydia trachomatis* as a possible agent of human autoimmune diseases. Microbiologica **12:**291–296.
14. **Goloubinoff, P., A. A. Gatenby, and G. H. Lorimer.** 1989. GroE heat-shock proteins promote assembly of foreign prokaryotic ribulose bisphosphate carboxylase oligomers in *Escherichia coli*. Nature (London) **337:**44–47.
15. **Grayston, J. T., S. Wang, L. Yeh, and C. Kuo.** 1985. Importance of reinfection in the pathogenesis of trachoma. Rev Infect. Dis. **7:**717-725.
16. **Hemmingsen, S. M., C. Woolford, S. M. van der Vies, K. Tilly, D. T. Dennis, C. P. Georgopoulos, R. W. Hendrix, and R. J. Ellis.** 1988. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature (London) **333:**330–334.

17. Holoshitz, J., F. Koning, J. E. Coligan, J. DeBruyn, and S. Strober. 1989. Isolation of CD4⁻CD8⁻ mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. Nature (London) 339:226–229.

18. Jindal, S., A. K. Dudani, B. Singh, C. B. Harley, and R. S. Gupta. 1989. Primary structure of a human mitochondrial protein homologous to the bacterial and plant chaperonins and to the 65-kilodalton mycobacterial antigen. Mol. Cell. Biol. 9:2279–2283.

19. Karn, J., S. Brenner, L. Barnett, and G. Cesaereni. 1980. Novel bacteriophage λ cloning vector. Proc. Natl. Acad. Sci. USA 77:5172–5176.

20. Kaufman, S. H. E. 1990. Heat shock proteins and the immune response. Immunol. Today 11:129–136.

21. Kaufman, S. H. E., U. Vath, J. E. R. Thole, J. D. A. Van Embden, and F. Emmrich. 1987. Enumeration of T cells reactive with Mycobacterium tuberculosis organisms and specific for the recombinant mycobacterial 64-kDa protein. Eur. J. Immunol. 17:351–357.

22. Keat, A. C., B. J. Thomas, D. Taylor, G. D. Pegrum, R. N. Maini, and J. T. Scott. 1980. Evidence of Chlamydia trachomatis infection in sexually acquired reactive arthritis. Ann. Rheum. Dis. 39:431–437.

23. Kingsbury, D. T., and E. Weiss. 1968. Lack of deoxyribonucleic acid homology between species of the genus Chlamydia. J. Bacteriol. 96:1421–1423.

24. Koga, T., A. Wand-Wurttenberger, J. DeBruyn, M. E. Munk, B. Schoel, and S. H. E. Kaufman. 1989. T cells against a bacterial heat shock protein recognize stressed macrophages. Science 245:1112–1115.

25. Kousa, M., P. Saikku, S. Richmond, and A. Lassus. 1978. Frequent association of chlamydial infection with Reiter's syndrome. Sex. Transm. Dis. 5:57–61.

26. Kuo, C. C., S. P. Wang, and J. T. Grayston. 1977. Growth of trachoma organisms in HeLa 229 cell culture, p. 328–336. In D. Hobson and K. K. Holmes (ed.), Nongonococcal urethritis and related infections. American Society for Microbiology, Washington, DC.

27. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680–685.

28. Lamb, J. R., V. Bal, P. Mendez-Samperino, A. Mehlert, A. So, J. Rothbard, S. Firdal, R. A. Young, and D. B. Young. 1989. Stress proteins may provide a link between the immune response to infection and autoimmunity. Int. Immunol. 1:191–196.

29. Lindquist, S., and E. A. Craig. 1988. The heat-shock proteins. Annu. Rev. Genet. 22:631–677.

30. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.

31. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

32. Mardh, D., J. Paavonen, and M. Puolakkainen. 1989. Chlamydia, p. 251–260. Plenum Medical Book Company, New York.

33. Menozzi, F. D., C. Menozzi-Dejaiffe, and F. E. Nano. 1989. Molecular cloning of a gene encoding a Chlamydia psittaci 57-kDa protein that shares antigenic determinants with ca. 60-kDa proteins present in many Gram-negative bacteria. FEMS Microbiol. Lett. 58:59–64.

34. Messing, J. 1983. New M13 vectors for cloning. Methods Enzymol. 101:20–78.

35. Morrison, R. P., R. J. Belland, K. Lyng, and H. Caldwell. 1989. Chlamydial disease pathogenesis. The 57-kD chlamydial hypersensitivity antigen is a stress response protein. J. Exp. Med. 170:1271–1283.

36. Morrison, R. P., K. Lyng, and H. D. Caldwell. 1989. Chlamydial disease pathogenesis. Ocular hypersensitivity elicited by a genus-specific 57-kD protein. J. Exp. Med. 169:663–675.

37. Morrison, R. P., H. Su, K. Lyng, and Y. Yuan. 1990. The Chlamydia trachomatis hyp operon is homologous to the groE stress response operon of Escherichia coli. Infect. Immun. 58:2701–2705.

38. Munk, M. E., B. Shoel, S. Modrow, R. W. Karr, R. A. Young, and S. H. E. Kaufman. 1989. T lymphocytes from healthy individuals with specificity to self epitopes shared by the mycobacterial and human 65-kilodalton heat shock protein. J. Immunol. 143:2844–2849.

39. Newhall, W. J. 1988. Macromolecular and antigenic composition of Chlamydiae, p. 47–70. In A. L. Barron (ed.), Microbiology of Chlamydia. CRC Press, Inc., Boca Raton, Fla.

40. Newhall, W. J., B. Batteiger, and R. B. Jones. 1982. Analysis of the human serological response to proteins of Chlamydia trachomatis. Infect. Immun. 38:1181–1189.

41. Platt, T. 1981. Termination of transcription and its regulation in the tryptophan operon of E. coli. Cell 24:10–23.

42. Pollack, J., J. N. Engel, E. Perara, and D. Ganem. 1990. The heat shock response of Chlamydia trachomatis, p. 137–140. In W. R. Bowie, H. D. Caldwell, R. P. Jones, P. A. Mardh, G. L. Ridgway, J. Schachter, W. E. Stamm, and M. E. Ward (ed.), Chlamydial infections. Cambridge University Press, Cambridge.

43. Ramanujam, R., G.-K. Sim, and A. Augustin. 1990. Self heat shock and γδ T cell reactivity. Proc. Natl. Acad. Sci. USA 87:1767–1771.

44. Reading, D. S., R. L. Hallberg, and A. M. Myers. 1989. Characterization of the yeast HSP-60 gene coding for a mitochondrial assembly factor. Nature (London) 337:655–659.

45. Res, P. C. M., C. G. Schaar, F. C. Breedveld, W. van Eden, J. D. A. van Embden, I. R. Cohen, and R. R. P. de Vries. 1988. Synovial fluid T cell reactivity against 65 kD heat shock protein of Mycobacteria in early chronic arthritis. Lancet ii:478–480.

46. Rothman, J. E. 1989. Polypeptide chain binding proteins: catalysis of protein folding and related processes in cells. Cell 59:591–601.

47. Shine, J., and L. Dalgarno. 1974. The 3'-terminal sequence of Escherichia coli 16S ribosomal RNA: complementary to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA 71:1342–1346.

48. Shinnick, T. M. 1987. The 65-kilodalton antigen of Mycobacterium tuberculosis. J. Bacteriol. 169:1080–1088.

49. Shinnick, T. M., M. H. Vodkin, and J. C. Williams. 1988. The Mycobacterium tuberculosis 65-kilodalton antigen is a heat shock protein which corresponds to common antigen and to the Escherichia coli groEL protein. Infect. Immun. 56:446–451.

50. Smith, G. B., and K. S. Johnson. 1988. Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. Gene 67:31–40.

51. Sompolinsky, D., J. B. Hertz, N. Hoiby, K. Jensen, B. Mansa, V. B. Pedersen, and Z. Samra. 1980. An antigen common to a wide range of bacteria. II. A biochemical study of a "common antigen" from Pseudomonas aeruginosa. Acta Pathol. Microbiol. Scand. Sect. B 88:253–260.

52. Sompolinsky, D., J. B. Hertz, N. Hoiby, K. Jensen, B. Mansa, and Z. Samra. 1980. An antigen common to a wide range of bacteria. I. The isolation of "common antigen" from Pseudomonas aeruginosa. Acta Pathol. Microbiol. Scand. Sect. B 88:143–149.

53. Stephens, R. S., E. A. Wagar, J. E. Allen, and P. Bavoil. 1990. Chlamydia trachomatis antigens in immunity and pathogenesis, p. 365–378. In N. Agabian and A. Cerami (ed.), Parasites: molecular biology, drug, and vaccine design. Wiley Liss, Inc., New York.

54. Taylor, H. R., S. L. Johnson, J. Schachter, H. D. Caldwell, and R. A. Prendergast. 1987. Pathogenesis of trachoma: the stimulus for inflammation. J. Immunol. 138:3023–3027.

55. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76:4350–4354.

56. Tsoulfa, G., G. A. W. Rook, G. M. Bahr, M. A. Sattar, K. Behbehani, D. B. Young, A. Mehlert, J. D. A. Van Embden, F. C. Hay, D. A. Isenberg, and P. M. Lydyard. 1989. Elevated IgG antibody levels to the Mycobacteria 65-kDa heat shock protein are characteristic of patients with rheumatoid arthritis. Scand. J. Immunol. 30:519–527.

57. van Eden, W., J. E. R. Thole, R. van der Zee, A. Noordzij, J. D. A. van Embden, E. J. Hensen, and I. R. Cohen. 1988. Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. Nature (London) 331:171–173.

58. Vilppula, A. H., U. I. Yli-Kerttula, A. K. Ahiroos, and P. E. Terho. 1981. Chlamydial isolations and serology in Reiter's syndrome. Scand. J. Rheumatol. 10:181–184.

59. Vodkin, M. H., and J. C. Williams. 1988. A heat shock operon in Coxiella burnetii produces a major antigen homologous to a protein in both mycobacteria and Escherichia coli. J. Bacteriol. 170:1227–1234.

60. Wagar, E. A., J. Schachter, P. Bavoil, and R. S. Stephens. 1990. Differential human serological response to two 60,000 molecular weight Chlamydia trachomatis antigens. J. Infect. Dis. 162:922–927.

61. Waldinger, D., C. Eckerskorn, F. Lottspeich, and H. Cleve. 1988. Amino-acid sequence homology of a polymorphic cellular protein from human lymphocytes and the chaperonins from Escherichia coli (groEL) and chloroplasts (Rubisco-binding protein). Biol. Chem. Hoppe-Seyler 369:1185–1189.

62. Watkins, N. G., W. J. Hadlow, A. B. Moos, and H. D. Caldwell. 1986. Ocular delayed hypersensitivity: a pathogenic mechanism of chlamydial conjunctivitis in guinea pigs. Proc. Natl. Acad. Sci. USA 83:7480–7484.

63. Young, D. B., J. Ivanyl, J. H. Cox, and J. R. Lamb. 1987. The 65kDa antigen of mycobacteria—a common bacterial protein? Immunol. Today 8:215–219.

64. Young, R. A., and T. J. Elliott. 1989. Stress proteins, infection, and immune surveillance. Cell 59:5–8.