The scrub typhus 58-kilodalton (kDa) antigen (Sta58) of Rickettsia tsutsugamushi is a major protein antigen often recognized by humans infected with scrub typhus rickettsiae. A 2.9 kilobase HindIII fragment containing a complete sta58 gene was cloned in Escherichia coli and found to express the entire Sta58 antigen and a smaller protein with an apparent molecular mass of 11 kDa (Stp11). DNA sequence analysis of the 2.9-kilobase HindIII fragment revealed two adjacent open reading frames encoding proteins of 11 (Stp11) and 60 kDa (Sta58). Comparisons of deduced amino acid sequences disclosed a high degree of homology between the R. tsutsugamushi proteins Stp11 and Sta58 and the E. coli proteins GroES and GroEL, respectively, and the family of primordial heat shock proteins designated Hsp10/Hsp60. Although the sequence homology between the Sta58 antigen and the Hsp60 protein family is striking, the Sta58 protein appeared to be antigenically distinct among a sample of other bacterial Hsp60 homologs, including the typhus group of rickettsiae. The antigenic uniqueness of the Sta58 antigen indicates that this protein may be a potentially protective antigen and a useful diagnostic reagent for scrub typhus fever.
Molecular Cloning and Sequence Analysis of the Sta58 Major Antigen Gene of *Rickettsia tsutsugamushi*: Sequence Homology and Antigenic Comparison of Sta58 to the 60-Kilodalton Family of Stress Proteins

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The scrub typhus 58-kilodalton (kDa) antigen (Sta58) of *Rickettsia tsutsugamushi* is a major protein antigen often recognized by humans infected with scrub typhus rickettsiae. A 2.9-kilobase HindIII fragment containing a complete *sta58* gene was cloned in *Escherichia coli* and found to express the entire Sta58 antigen and a smaller protein with an apparent molecular mass of 11 kDa (Stp11). DNA sequence analysis of the 2.9-kilobase HindIII fragment revealed two adjacent open reading frames encoding proteins of 11 (Stp11) and 60 (Sta58) kDa. Comparisons of deduced amino acid sequences disclosed a high degree of homology between the *R. tsutsugamushi* proteins Stp11 and Sta58 and the *E. coli* proteins GroES and GroEL, respectively, and the family of primordial heat shock proteins designated Hsp10 Hsp60. Although the sequence homology between the Sta58 antigen and the Hsp60 protein family is striking, the Sta58 protein appeared to be antigenically distinct among a sample of other bacterial Hsp60 homologs, including the typhus group of rickettsiae. The antigenic uniqueness of the Sta58 antigen indicates that this protein may be a potentially protective antigen and a useful diagnostic reagent for scrub typhus fever.

*Rickettsia tsutsugamushi*, the causative agent of the sometimes fatal febrile illness known as scrub typhus, is an obligate intracellular bacterium that is transmitted to humans by the mite vector *Leptotrombidium deliense* (52). In humans, immunity after infection lasts for 1 to 3 years against the homologous strain but is short lived (less than 1 year) against heterologous strains (46, 47). Studies with laboratory animals have shown that both cellular and humoral immune responses occur after infection, with the cellular immune response being required for protection (16, 19, 25, 42). Humans infected with scrub typhus rickettsiae produce serum antibodies to at least eight rickettsial proteins, including the 110-, 58-, 56-, and 47-kilodalton (kDa) polypeptides (Karp strain; E. V. Oaks, unpublished data). Of these scrub typhus antigens (Sta), the 56-kDa major outer membrane protein (Sta56) and the 58-kDa protein (Sta58) are among the most abundant proteins in this rickettsia and are the antigens most often recognized by infected animals and humans (13, 51, 53; E. V. Oaks, unpublished data). At least two antigens (Sta110 and Sta56) have strain-variable molecular weights, contain strain-specific epitopes, and are possibly involved in the strain-specific protective immune response (10, 13, 32, 51). The Sta58 antigen appears to be more highly conserved among scrub typhus rickettsiae, since all strains tested to date express indistinguishable epitopes on a polypeptide that consistently runs at an apparent molecular mass of 58 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (32).

Because sufficient quantities of this intracellular pathogen are difficult to obtain, the genes encoding all the major antigens and some of the minor antigens of *R. tsutsugamushi* have been cloned and expressed in *Escherichia coli* to facilitate the evaluation of these potentially protective antigens as components of a subunit vaccine and future diagnostic procedures (32, 33, 50). In this paper, we report the molecular cloning and expression of the gene encoding the Sta58 protein antigen in *E. coli*. DNA sequence analysis of a 2.9-kilobase (kb) HindIII fragment carrying the Sta58 gene revealed two adjacent open reading frames (ORFs) potentially encoding scrub typhus proteins of 11 and 60 kDa (stp11 and sta58, respectively). The deduced amino acid sequence of the *sta58* ORF was found to be homologous to those of the bacterial common antigens and the highly conserved family of 60-kDa heat shock proteins known collectively as Hsp60 stress proteins (15, 27, 41, 48, 56).

The Hsp60 homologs from a number of microbial pathogens are being evaluated as potential vaccine components, in part because they are abundant proteins and are usually recognized by the immune system of the infected host (39, 55, 56). Similarly, the Sta58 protein is one of the most abundant proteins in *R. tsutsugamushi* that is also immunogenic. The Hsp60 protein family exhibits a high degree of homology and antigenic cross-reactivity, with very few unique determinants having been described (15, 41, 48, 56). The high degree of conservation of Hsp60 protein subunits could potentially trigger an autoimmune response to the human Hsp60 homolog (54). In this study, we demonstrate that a substantial degree of heterogeneity is also possible within the highly homologous framework of the Hsp60 family, as only weak serological cross-reactivity was observed between the Sta58 antigen and the Hsp60 homologs of some other common bacterial pathogens. A surprising lack of antigenic similarity between the Sta58 protein and the Hsp60 homolog of *Rickettsia typhi* may also indicate that *R.
tsutsugamushi is more distantly related to the other members of the genus Rickettsia than was previously thought.

**MATERIALS AND METHODS**

**Bacterial strains, media, and passage preparation of R. tsutsugamushi.** A plaque-purified Karp strain of *R. tsutsugamushi* was used throughout this study. The passage history of this strain was embryonated chicken eggs (52 times). L cells (3 times), and embryonated chicken eggs (6 times). Rickettsiae were grown in mouse fibroblasts (L-929 cells) incubated at 34°C in a humidified atmosphere of 5% CO₂–95% air. The rickettsiae were harvested 5 to 7 days post-infection and subsequently purified on Renografin (Squibb Diagnostics, New Brunswick, N.J.) density gradients as previously described (33). *E. coli* host strains and vectors are described below. All *E. coli* strains were grown in Luria broth or Luria broth agar (24). Luria broth was supplemented with ampicillin (100 μg/ml) for *E. coli* containing all plasmid pBR322 derivatives.

**Recombinant DNA procedures.** *R. tsutsugamushi* genomic DNA was isolated from purified rickettsiae as previously described (33). All restriction enzymes, T4 DNA ligase, and bacterial alkaline phosphatase were obtained from New England BioLabs (Beverly, Mass.) and used per the manufacturer’s instructions. *Agt11* libraries were constructed in *E. coli* host strain Y1090 (57) by using a modified method with conversion adaptor oligonucleotides as described by Stover et al. (50). Antigen-positive recombinant bacteriophage were identified by screening with hyperimmune rabbit sera (33, 50). Two recombinant phages (agt11Rts224 and agt11Rts408) expressing Sta58 epitopes were used as a source of DNA for probing plasmid libraries. Recombinant agt11 phage DNA was purified as described by Silhavy et al. (44). Cloned inserts were separated from recombinant agt11 phage DNA by EcoRI digestion and agarose gel electrophoresis on 0.5% low-melting-point agarose (International Biotechnologies, Inc., New Haven, Conn.). Restriction fragment bands were excised, melted at 60°C in 50 mM Tris (pH 8.0)–100 mM NaCl buffer, and purified by using Elu-Tips (Schleicher & Schuell, Inc., Keene, N.H.). Plasmid DNA was prepared by a modification (26) of the procedure described by Birnboim and Doly (3). Plasmid DNA was transformed into *E. coli* HB101 competent cells by using the manufacturer’s specifications (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). DNA was visualized with ethidium bromide as previously described (4, 23, 31). Staphylococcal protein A cations ([a-35S]methionine assay medium (Difco). The labeled maxicell preparations were analyzed by discontinuous 13% SDS-polyacrylamide gel electrophoresis (31) with the buffers described by Laemmli (23). Autoradiography using Kodak Blue Brand X-ray film was performed on the separated proteins after they were electrophoblated onto nitrocellulose.

**Protein electrophoresis and Western blotting.** Rabbit anti-*R. tsutsugamushi* antisera was prepared from a rabbit inoculated with gradient-purified whole *R. tsutsugamushi* Karp, which was also used for Western blot (immunoblot) analysis. This serum was exhaustively absorbed with *E. coli* for use in Western blot analysis of recombinant *E. coli* as described previously (33). Anti-BCG serum was raised in BALB/c mice to a cell wall fraction prepared from *Mycobacterium bovis* BCG (bacillus Calmette-Guérin) (Ribi Immunocore Research, Inc., Hamilton, Mont.). Polyclonal antisera to the *Coxiella burnetii* HtpB protein (Hsp60 homolog) was raised to a peptidoglycan fraction from a recombinant *E. coli* strain expressing the *C. burnetii* HtpB antigen as described by Vodkin and Williams (55). The Nine Mile strain of *C. burnetii* was grown and purified as described by Vodkin and Williams (55). The BCG cell wall fraction, purified *C. burnetii* Nine Mile, and antiserum to the BCG cell wall fraction and *C. burnetii* HtpB were kindly provided by Jim C. Williams, U.S. Army Research Institute of Infectious Diseases, Frederick, Md. SDS-PAGE and Western blotting of rickettsial polypeptides were performed as previously described (4, 23, 31). Staphylococcal protein A conjugated with alkaline phosphatase (Cappel, Organon Teknika Corp., West Chester, Pa.) was used to detect the antibody bound to antigens in the Western blot assay. Alkaline phosphatase-conjugated probes were developed with fast red TR salt and naphthol AS-MX phosphate as previously described (43).

**RESULTS**

**Molecular cloning of a complete sta58 gene.** Fragments of the gene encoding the 58-kDa protein antigen of *R. tsutsugamushi* had been previously cloned in agt11 (32, 50). Purified restriction fragment inserts from agt11 clones (clones agt11Rts224 and agt11Rts408) expressing antigenic determinants of the Sta58 protein were used as nucleic acid probes. This serum was exhaustively absorbed with *E. coli* for use in Western blot analysis of recombinant *E. coli* as described previously (33). Anti-BCG serum was raised in BALB/c mice to a cell wall fraction prepared from *Mycobacterium bovis* BCG (bacillus Calmette-Guérin) (Ribi Immunocore Research, Inc., Hamilton, Mont.). Polyclonal antisera to the *Coxiella burnetii* HtpB protein (Hsp60 homolog) was raised to a peptidoglycan fraction from a recombinant *E. coli* strain expressing the *C. burnetii* HtpB antigen as described by Vodkin and Williams (55). The Nine Mile strain of *C. burnetii* was grown and purified as described by Vodkin and Williams (55). The BCG cell wall fraction, purified *C. burnetii* Nine Mile, and antiserum to the BCG cell wall fraction and *C. burnetii* HtpB were kindly provided by Jim C. Williams, U.S. Army Research Institute of Infectious Diseases, Frederick, Md. SDS-PAGE and Western blotting of rickettsial polypeptides were performed as previously described (4, 23, 31). Staphylococcal protein A conjugated with alkaline phosphatase (Cappel, Organon Teknika Corp., West Chester, Pa.) was used to detect the antibody bound to antigens in the Western blot assay. Alkaline phosphatase-conjugated probes were developed with fast red TR salt and naphthol AS-MX phosphate as previously described (43).
The 33% G+C content of the sequenced 2.9-kb HindIII fragment carrying the gene for the Sta58 protein was subjected to DNA sequence analysis (Fig. 2). The recombinant and native Sta58 antigens of R. tsutsugamushi are indicated in lanes 3 and 4, respectively. The right is an autoradiograph of [35S]methionine-labeled maxicell preparations of HB101 (pBK322) (lane 5) and HB101/pRTS58H2.9. (lane 6) separated by SDS-PAGE. The Sta58 antigen and Stp11 protein, both encoded by pRTS58H2.9, are indicated in lane 6 of the maxicell SDS-PAGE autoradiograph. Stp11 was not recognized by the serum used in the Western blot (lane 3). The β-lactamase protein encoded by the pBR322 vector (β-La) is indicated in lanes 5 and 6 of the maxicell preparations.

Acid probes to identify restriction fragments associated with the sta58 gene by Southern blot hybridization (data not shown). A 2.9-kb HindIII fragment, identified in this manner, was cloned into the HindIII site of pBR322 and identified by colony blot in situ hybridization with the sta58 nucleic acid probes. The resulting plasmid, designated pRTS58H2.9, was found to express proteins with apparent molecular masses of 11 and 58 kDa by SDS-PAGE maxicell analysis (Fig. 1). Western blot analysis showed that the recombinant 58-kDa protein reacted with rabbit hyperimmune anti-R. tsutsugamushi serum that had been preabsorbed with E. coli. The recombinant protein comigrated with the 58-kDa antigen of R. tsutsugamushi (Fig. 1, lanes 3 and 4). The small apparent molecular mass difference between the recombinant Sta58 antigen and the Sta56 strain-specific major antigen of R. tsutsugamushi (strain Karp) could also be demonstrated (Fig. 1, lanes 2 and 4 [recombinant and native Sta56, respectively]). The complete recombinant 58-kDa protein antigen also reacted with anti-Sta58 serum that had been affinity purified by using recombinant antigen from agt11 clones expressing smaller antigenic fragments of the Sta58 antigen (data not shown). The smaller 11-kDa recombinant protein did not react with the rabbit anti-R. tsutsugamushi serum by Western blot analysis.

Sequence analysis of the DNA encoding Stp11 and Sta58. The 2.9-kb HindIII fragment carrying the gene for the Sta58 protein was subjected to DNA sequence analysis (Fig. 2). The 33% G+C content of the sequenced 2.9-kb HindIII restriction fragment is comparable with the R. tsutsugamushi genomic G+C content of 35% (C. K. Stover, unpublished data). Translations of all possible reading frames revealed two ORFs separated by 72 bases (Fig. 2). These two ORFs, designated stp11 and sta58, were only marginally less AT-rich (36% G+C) than the entire 2.9-kb HindIII fragment. The 11- and 60-kDa coding capacities of these two ORFs were in reasonable agreement with the sizes of the recombinant 11- and 58-kDa gene products identified by maxicell analysis. Polyuridine-rich potential ribosomal binding sites were located 4 bases from the start codon of the stp11 ORF and 8 bases from the start codon of the sta58 ORF. Reasonable matches to the E. coli promoter –10 and –35 consensus sequences were also found 110 bases 5’ from the likely start codon of the sta11 gene (Fig. 2).

Homology searches using the derived amino acid sequence encoded by the sta58 ORF disclosed striking identity to the E. coli GroEL protein, the mycobacterial 65-kDa antigens, the C. burnetii HtpB protein, and the large family of 60-kDa stress proteins (data not shown) (15, 41, 45, 56, 58). Figure 3 depicts a best-fit amino acid sequence alignment of the R. tsutsugamushi Sta58 protein and the published sequences of other Hsp60 proteins from four bacterial genera, one plant, one yeast, and humans (14, 17, 28, 35, 55). The amino acid sequence homology between the deduced Sta58 protein and the Hsp60 family averaged 49% identical residues (Fig. 3).

Alignment of hydrophobicity curves for each of the seven Hsp60 homologs disclosed superimposable hydrophobic profiles, indicating that a substantial percentage of the nonidentical amino acids are of conserved type. The alignment of the seven Hsp60 sequences revealed at least four hyperconserved regions along the Hsp60 poly peptide backbone. The C-terminal region of the Sta58 sequence contained the same repetitive Gly-Gly-Met motif observed in all Hsp60 homologs except the plant Triticum aestivum (wheat) (14). It has been suggested that the hydrophobic C-terminal region of this protein family is transmembraneous and that the hydrophilic repetitive Gly-Gly-Met sequence at the extreme C terminus may serve to anchor this protein.

Previous sequence analysis of regions encoding the Hsp60 homologs of E. coli (GroEL), C. burnetii (HtpP), and Synechococcus strain 6301 (HtpA) and the large family of stress proteins encoding a 10- to 11-kDa protein 5’ to, and probably cotranscribed with, the hsp60 gene (7, 14, 55). These smaller 10- to 12-kDa polypeptides are designated Hsp10 proteins. Computer-assisted best-fit alignments between the derived amino acid sequence of the recombinant R. tsutsugamushi Stp11 protein and the derived amino acid sequences of E. coli GroES, C. burnetii HtpA, Mycobacterium tuberculosis 12-kDa antigen, and Synechococcus strain 6301 Ur3 disclosed an average sequence identity of 46%. As in the comparisons of Hsp60 sequences, a substantial percentage of the nonidentical amino acids were similar, resulting in generally superimposable hydrophobic profiles (Fig. 4). Homology between four bacterial GroES homologs was found to be uniform throughout the length of the relatively small polypeptide backbone.

Other studies have shown that the E. coli stress protein GroEL and the Hsp60 homologs of C. burnetii (HtpP), M. tuberculosis, and Mycobacterium smegmatis (65-kDa antigen) are heat shock proteins (29, 41, 55). Potential Eor3 heat shock promoter –10 and –35 regions have been found 5’ to the groES-groEL gene operon of E. coli and the htpA-htpB operon of C. burnetii (6, 14, 55). Expression of the recom
Antigenic uniqueness of Sta58 among the family of Hsp60 stress proteins. The first evidence of a 60-kDa common antigen family (Hsp60) among procaryotes was based on serum cross-reactivity (15). Antigenic cross-reactivity between the *R. tsutsugamushi* Sta58 and the Hsp60 common antigens has not been previously reported. Because of the strong sequence homology observed between the *R. tsutsugamushi* Sta58 protein and the Hsp60 family of stress proteins, the Sta58 antigen was compared with other bacterial Hsp60 homologs by Western blot analysis with polyvalent sera (Fig. 3). The similarity in amino acid sequence between the Sta58 and the Hsp60 antigens is indicated by the presence of amino acid residues identical to those in the Hsp60 family (Fig. 4).
observed with five different monoclonal antibodies to the purified R. typhi Hsp60 antigen. Two of these monoclonal antibodies are shown in Fig. 3. None of these five monoclonal antibodies exhibited reactivity with the R. tsutsugamushi Sta58 protein. Cross-reactivity was observed between the C. burnetii and R. tsutsugamushi sera and the respective Hsp60 homologs of these two bacteria, but this cross-reactivity was substantially less than that observed in all other cases.

**DISCUSSION**

The Sta58 protein, which is most likely the 63-kDa protein described by Hanson (12, 13) and the 60-kDa protein described by Tamura et al. (51), is an abundant protein of R. tsutsugamushi and is often recognized by animals and humans infected with scrub typhus rickettsiae. The cloning and
expression of the *R. tsutsugamushi* sta58 gene in *E. coli* will facilitate further evaluation of the Sta58 protein as a possible component of future scrub typhus subunit vaccines. On the basis of overwhelming amino acid sequence identity (average, 49%) and similarity we conclude that the Sta58 protein antigen is a member of the Hsp60 family of stress proteins, also termed common antigens. However, among the complete bacterial Hsp60 sequences known, the *R. tsutsugamushi* Sta58 antigen appears to be the least similar. In contrast to the observed genetic relatedness of Sta58 and Hsp60 proteins, Western blot analysis of a number of bacteria with a variety of polyclonal and monoclonal antisera (known to react with Hsp60 proteins) indicated that the *R. tsutsugamushi* Sta58 protein is also unique antigenically. Although observations, including distinct ultrastructure and unique physiological requirements, have suggested that *R. tsutsugamushi* is clearly unique among other members of the genus *Rickettsia* (21, 45), it was unexpected that we would find a lack of antigenic similarity between the *R. tsutsugamushi* Sta58 antigen and the Hsp60 homolog of *R. prowazekii*, a member of the typhus group of rickettsiae. This result is particularly surprising because substantial antigenic similarity was observed between *Rickettsia prowazekii* and the Hsp60 homologs of other, presumably less-related bacteria. It should be noted that this study used a set of five monoclonal antibodies (including those shown in

FIG. 4. Comparison of Hsp10 and Hsp60 hydrophobicity profiles. Hydrophobicity was plotted by the method of Kyte and Doolittle (22). Regions above the horizontal axis are hydrophobic. Abbreviations are defined in the legend to Fig. 3.
protein, even though the genus Coxiella is in the tribe Rickettsiaceae and was once in the genus Rickettsia (8).

Because it is believed that the Hsp10 proteins and Hsp60 proteins complex with each other, it is possible that the stop11 and sta58 genes are cotranscribed or coregulated (14). Before this study, the genes encoding Hsp10 and Hsp60 protein homologs had been found to be closely linked, and probably cotranscribed, from hsp10 to hsp60 in E. coli, C. burnetii, and Synechococcus strain 6301 (14, 55). In this study, the location of promoterlike sequences to the stop11 gene, the apparent absence of promoterlike sequences for the adjacent downstream sta58 gene, and the lack of recognizable transcriptional terminators between the two genes suggests that the stop11 and sta58 genes may be cotranscribed in the same order as the hsp10 and hsp60 genes of E. coli, C. burnetii, and Synechococcus strain 6301. Exceptions to the close gene linkage include the hsp10 (BCG-a gene) and hsp60 genes of M. tuberculosis (40) and genes encoding Hsp60 homologs from a number of plants and other eucaryotes, including humans (5, 14, 17).

One of the eventual goals of this effort is to evaluate the R. tsutsugamushi Sta58 major protein antigen as a potentially protective antigen. Because the Hsp60 proteins are generally immunodominant antigens, a number of groups are also studying the Hsp60 proteins of other procaryotic and eucaryotic pathogens as protective antigens (39, 55, 56). A major concern regarding the use of an Hsp60 antigen or any other highly conserved protein in a vaccine is the potential for the antigen to elicit an autoimmune response to a homologous self protein. A number of bacterial pathogens have been associated with autoimmune disorders (including arthritis). In the case of the rat adjuvant arthritis model, arthritogenic T cells have been shown to cross-react with the M. tuberculosis 65-kDa antigen and an unidentified protein in synovial fluid, but it remains to be determined whether this cross-reactivity is due to a specific immune response to the Hsp60 of the pathogen. Recent evidence has prompted the hypothesis that the immune system is actually predisposed to autoreact with self Hsp60 (and possibly other highly conserved stress proteins) and that this autoimmune response is central to a normal mechanism for immune surveillance (20, 30, 34; for a review, see reference 58).

Alternatively, it has been questioned whether the highly homologous stress proteins could have any protective vaccine potential at all, since infection by one pathogen does not normally protect an individual from infection with a different pathogen. It has been suggested by Young et al. (56) that exposure to bacterial stress protein families early in life may induce immunity to a variety of different pathogens and could explain why only a fraction of individuals infected with some pathogens actually acquire clinical disease. It is our belief that further study of the R. tsutsugamushi Sta58 antigen and Hsp60 proteins as protective antigens is warranted because it is clear from previous studies and this study that within the highly homologous framework of the Hsp60 proteins there is a considerable capacity for unique antigenic determinants and, therefore, the possibility for specific protective immunity. Because lasting protection against scrub typhus rickettsiae is strain specific, the Sta58 antigen by itself may not be sufficient for a protective subunit vaccine, as no apparent strain-specific differences in the R. tsutsugamushi Sta58 antigen have been observed to date (32). Nevertheless, initial short-term heterologous protection may indicate that an antigen or antigens common to the strains of R. tsutsugamushi might provide marginal short-term protection. It is also possible that marginally protective

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**Fig. 5.** Serological comparison of Hsp60 common antigen homologs by Western blot analysis. Various antigen preparations (R. tsus., R. tsutsugamushi; C. bur., C. burnetii; R. pro., R. prowazekii; S. lors., Shigella flexnerii; BCG, M. bovis BCG; N. men., Neisseria meningitidis) were electrophoresed and electroeluted onto nitrocellulose. Each panel represents a similar gel with each battery of antigens. Only the portion of the gel containing the Hsp60 antigens is shown. Each antigen group was reacted separately with the antisera designated on the left (a, polyclonal antiserum to gradient-purified R. tsutsugamushi HtpB antigen; b, polyclonal antiserum to recombinant C. burnetii HtpB protein [55]; c, polyclonal antiserum to BCG cell wall fraction; d, monoclonal antiserum TC30-2F2.2 to purified R. typhi Hsp60; e, monoclonal antiserum T5-1F1.5 to purified R. typhi Hsp60).
antigen components are necessary for a complete and effective vaccine.

In addition to their potential as components in protective vaccines, recombinant Hsp60 homologs from a variety of organisms may be useful for the stability and correct assembly of recombinant foreign proteins. While the Hsp60 proteins probably carry out similar functions in their respective hosts, there are some indications that they are not completely interchangeable and that Hsp60 proteins may have some preference for proteins of their normal environment. For example, a temperature-sensitive *E. coli* GroEL mutant could not be suppressed by a recombinant *M. tuberculosis* homolog (41). It has also been demonstrated that hyperexpression of a nonnative Hsp10-Hsp60 can permit the correct assembly of an enzyme complex that normally requires the Karp, Gilliam, and Kato strains of the genes for ATP synthase subunits in the cyanobacterium *Synechococcus* 6301. J. Mol. Biol. 194:359-383.


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