INFECTION AND IMMUNITY, Oct. 1989, p. 3116–3122
0019-9567/89/103116-07$02.00/0

Vol. 57, No. 10

Antigenic and Genetic Relatedness of Eight *Rickettsia tsutsugamushi* Antigens

EDWIN V. OAKS,[1]* ROBERT M. RICE,[1]† DARYL J. KELLY,[2]‡ AND C. KEN STOVER[1]

Department of Rickettsial Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20307,[1] and U.S. Army Medical Research Unit, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia[2]

Received 18 April 1989/Accepted 11 July 1989

The genetic and antigenic relatededness of eight antigens in three strains of *Rickettsia tsutsugamushi* has been studied by using recombinant organisms expressing epitopes of the 150-, 110-, 72-, 58-, 56-, 49-, 47-, and 20-kilodalton (kDa) polypeptide antigens of the Karp strain. Southern blot analysis of Karp, Kato, and Gilliam strain genomic DNA by using probes specific for each antigen class indicated that while strong homology exists between each of the corresponding antigen genes in these three strains, some restriction fragment length polymorphism exists. Antibodies affinity purified against each recombinant antigen class reacted with a comparably sized polypeptide in the Karp, Kato, and Gilliam strains in Western blots (immunoblots). Against more recent human isolates of *R. tsutsugamushi*, the affinity-purified antibodies against the 58-kDa recombinant antigen (anti-58-kDa) reacted with all nine isolates, anti-56-kDa reacted with eight of nine isolates, anti-47-kDa reacted with eight of nine isolates, anti-72-kDa reacted with eight of nine isolates, and anti-110-kDa reacted with four of nine isolates. Additional analysis indicated that the 110-kDa antigen may contain strain-specific epitopes similar to those previously reported for the 56-kDa polypeptide. Evidently, the strain heterogeneity among scrub typhus rickettsiae is a result of multiple components that exhibit variability in a background of strong homology.

Rickettsia tsutsugamushi, the causative agent of tsutsugamushi disease or scrub typhus fever, is an obligate intracellular bacterium. Unlike the typhus and spotted fever groups of rickettsiae, for which biological, serological (26), and DNA homology (10, 11) studies have been used to demonstrate relationships among members, only a serological analysis has been possible for the scrub typhus group. This is largely because of technical problems in growing and purifying large quantities of *R. tsutsugamushi* for DNA homology studies. The serological analysis, along with certain biological properties relating primarily to virulence and cross-protection in laboratory animals, has clearly demonstrated that the scrub typhus group consists of a multitude of strains, with the reference strains being the Karp, Kato, and Gilliam strains. These *R. tsutsugamushi* strains have both serologically unique epitopes and many cross-reactive epitopes (2, 5, 6, 18, 19, 27). Little, if any, demonstrable serological cross-reactivity exists between scrub typhus rickettsiae and members of the typhus or spotted fever groups of rickettsiae. Recently, it has been demonstrated with monoclonal antibodies that one class of *R. tsutsugamushi* envelope proteins contains strain-specific epitopes (5, 7, 23). This family of polypeptides exhibits unique apparent molecular sizes in the Karp (56-kilodalton [kDa]), Kato (54-kDa), and Gilliam (57-kDa) strains supporting the presence of strain-specific regions in the polypeptide (7, 14, 23). Group-reactive epitopes are also present on these polypeptides (5, 7, 14, 23), suggesting that the scrub typhus antigen genes (*sta*) encoding these polypeptides contain regions of high similarity and also regions of less similarity (i.e., strain specific). The only other polypeptide with strain-specific characteristics (similar to the 56-kDa polypeptide group) with respect to its apparent size in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels is the 110-kDa polypeptide (14), but strain-specific epitopes have not been identified on this particular polypeptide. Other polypeptide antigens of *R. tsutsugamushi* have been described, including the 150-, 72-, 58-, 49-, 47-, and 20-kDa polypeptides, but the interstrain relatedness of these antigens and their genes is not known.

Recently, we reported that eight different *R. tsutsugamushi* (strain Karp) genes encoding distinct antigens have been cloned into *Escherichia coli* by using the λgt11 vector (14, 22). The availability of these recombinants, which express scrub typhus rickettsiae polypeptide epitopes, has allowed the analysis of *R. tsutsugamushi* genes and gene products. In this study, DNA isolated from these clones was used to test for homologous antigen genes and gene stability in the Karp, Kato, and Gilliam strains. In addition, antibodies affinity purified against each recombinant product were used to determine the antigenic relatedness of each antigen among the three reference strains. The antigenic analysis was extended to recent human isolates to determine whether epitopes present in the long-passaged reference Karp strain are still present in low-passage scrub typhus isolates.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Three plaque-purified reference strains (Karp, Kato, and Gilliam) of *R. tsutsugamushi* were used throughout this study. The passage histories of these rickettsial seeds were as follows: Karp strain, passaged 52 times in embryonated chicken eggs, 3 times in mouse L-929 cells, and 6 times in embryonated chicken eggs (E52-L3-E6); Kato, E162-L3-E8; and Gilliam, E164-L3-E8. Rickettsiae were grown in mouse fibroblasts (L-929 cells) incubated at 34°C in a humidified atmosphere of 5% CO_2–95% air. The rickettsiae were harvested 5 to 7 days

* Corresponding author.
† Present address: U.S. Army Research and Development Command, Division of Biological Systems, Fort Detrick, MD 21701.
‡ Present address: Division of Rickettsial Diseases, Naval Medical Research Institute, Bethesda, MD 20814.

TABLE 1. Characteristics of λgt11 recombinant phage containing *R. tsutsugamushi* DNA inserts

Clone[a]	Antigen gene	Probe DNA insert size[b]	Lysogen product[c]
λgt11Rts201	*sta*20	750	20 kDa
λgt11Rts401	*sta*47	2,500	47 kDa
λgt11Rts307	*sta*49	4,100	43 kDa
λgt11Rts12	*sta*56	700	FP
λgt11Rts224	*sta*58	1,150	FP
λgt11Rts30	*sta*72	1,000	FP
λgt11Rts14	*sta*110	5,400	110 kDa
λgt11Rts407	*sta*110	1,350	FP
λgt11Rts24	*sta*110	3,700	FP
λgt11Rts33	*sta*150	1,000	FP

[a] The clones listed were constructed as described by Stover et al. (22). Clones λgt11Rts12 and λgt11Rts14 have been previously described (14).

[b] DNA insert sizes are given in base pairs.

[c] Lysogen product refers to the size of the protein antigen synthesized by a lysogen which was reactive with rabbit anti-Karp sera. The apparent size was determined by SDS-PAGE and Western blot. FP indicates that the reactive lysogen product was a fusion protein in that it reacted with both rabbit anti-Karp and rabbit anti-β-galactosidase. The apparent molecular sizes of the fusion proteins were all greater than 116 kDa, the size of unfused β-galactosidase.

postinfection and subsequently purified on Renografin (Squibb Diagnostics, New Brunswick, N.J.) density gradients as previously described (14).

Recent *R. tsutsugamushi* isolates were obtained from febrile patients attending the Mentekab district hospital in Pahang, Malaysia (3). These isolates were passaged three to four times in adult ICR mice and two times in L cells prior to Western blot (immunoblot) analysis (see below).

Recombinant DNA methods. *R. tsutsugamushi* genomic DNA was isolated from purified rickettsiae as previously described (14). λgt11 libraries were constructed with conversion adaptor oligonucleotides as described by Stover et al. (22). *E. coli* Y1090 and Y1089 were used for λgt11 library construction and maintenance of recombinants (28). Table 1 lists the recombinant strains used in this study. Cloned *R. tsutsugamushi* DNA was isolated from *E. coli* Y1089 (28) lysogens carrying the recombinant bacteriophage. The lysogen cultures were grown at 37°C in Luria broth containing ampicillin (100 μg/ml) to an A_{650} of 0.4 and then shifted to 42°C for 30 min as previously described (14) and treated with NaCl and chloroform at 2 to 3 h postinduction with isopropylthiogalactoside (IPTG). Recombinant λgt11 phage were purified from *E. coli* Y1089 lysogens by polyethylene glycol precipitation and glycerol gradient purification (9). Recombinant λgt11 phage DNA was isolated from the purified phage by phenol-chloroform extraction and ethanol precipitation as previously described (9). Cloned inserts were separated from recombinant λgt11 phage DNA by *Eco*RI digestion and agarose gel electrophoresis on 5% low-melting-point agarose (International Biotechnologies, Inc., New Haven, Conn.). The restriction fragment band was 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.) by the manufacturer's specifications.

Southern blot analysis of *R. tsutsugamushi* genomic DNA. *R. tsutsugamushi* genomic DNA from the Karp, Kato, and Gilliam strains was digested with either *Sau*3A or *Taq*I restriction enzyme (New England BioLabs, Inc., Beverly, Mass.) prior to electrophoresis in 1.2% agarose gels. After transfer to nitrocellulose (Schleicher & Schuell), the rickettsial DNA was probed with ^{32}P-labeled DNA derived from λgt11 recombinants containing various inserts for different antigen genes of *R. tsutsugamushi* (Table 1). Purified restriction fragments were labeled with Dupont NEN [α-^{32}P]dCTP with a commercial nick translation kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). Southern blot hybridization was performed as described by Maniatis et al. (9) under stringent hybridization (35°C in 50% formamide–6× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]–2× Denhardt solution) and washing (62°C, 100 mM NaCl–0.1% SDS) conditions (9). The fragments were autoradiographed by using Kodak Blue Brand Film and X-ray film cassettes with intensifying screens at −70°C.

Analysis of rickettsial antigens expressed in *E. coli* and *R. tsutsugamushi*. To identify the antigen gene cloned into λgt11, affinity-purified antibodies were prepared against each recombinant and subsequently used in Western blots against *R. tsutsugamushi* to determine which rickettsial antigen was being expressed by the recombinants. Pooled rabbit anti-*R. tsutsugamushi* (Karp strain) sera exhaustively absorbed (14) with Y1089 and λgt11 was incubated with IPTG-saturated nitrocellulose filters (diameter, 137 mm) containing approximately 2×10^5 recombinant plaques. The bound antibodies were eluted with a glycine (pH 2.8) buffer, diluted (1:3) in casein filler, and used to probe rickettsial polypeptides in a Western blot procedure as previously described (14). Staphylococcal protein A (Pharmacia, Piscataway, N.J.) radioiodinated by the chloramine T method (8) or 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 (Sigma Chemical Co., St. Louis, Mo.) (20).

Lysogens expressing rickettsial antigens were also analyzed by Western blots with the absorbed rabbit anti-*R. tsutsugamushi* sera to determine whether the cloned gene product was under the regulation of the *lac* promoter (i.e., inducible with IPTG) and to determine the apparent molecular size of the expressed recombinant protein.

Direct fluorescent-antibody (DFA) analysis of recent human isolates of *R. tsutsugamushi* was performed as previously described (18).

Protein electrophoresis and Western blotting. SDS-PAGE and Western blotting of rickettsial polypeptides were performed as described by Oaks (13). The recent human isolates were not purified prior to SDS-PAGE; rather, infected L cells were solubilized directly in electrophoresis sample buffer (12), heated to 100°C for 5 min, and stored at −80°C until electrophoresis was performed.

RESULTS

Recombinants expressing *R. tsutsugamushi* polypeptide epitopes. Previously, we reported that several unique antigen genes (including the genes encoding the 150-, 110-, 72-, 58-, 56-, and 49-kDa polypeptides) from the Karp strain of *R. tsutsugamushi* were cloned into *E. coli* by using a modification of the λgt11 cloning method (22). λgt11 recombinants expressing determinants of two additional antigens of 20 and 47 kDa were also obtained from the same λgt11 clone bank, resulting in a collection of λgt11 recombinants carrying gene segments of eight different antigen genes. In each case, the cloned antigen gene was identified by using antibodies affinity purified against the recombinant to determine, by Western blotting against *R. tsutsugamushi*, which native rickettsial polypeptide shared epitopes with the recombinant polypeptide. In this paper, representative clones of each of

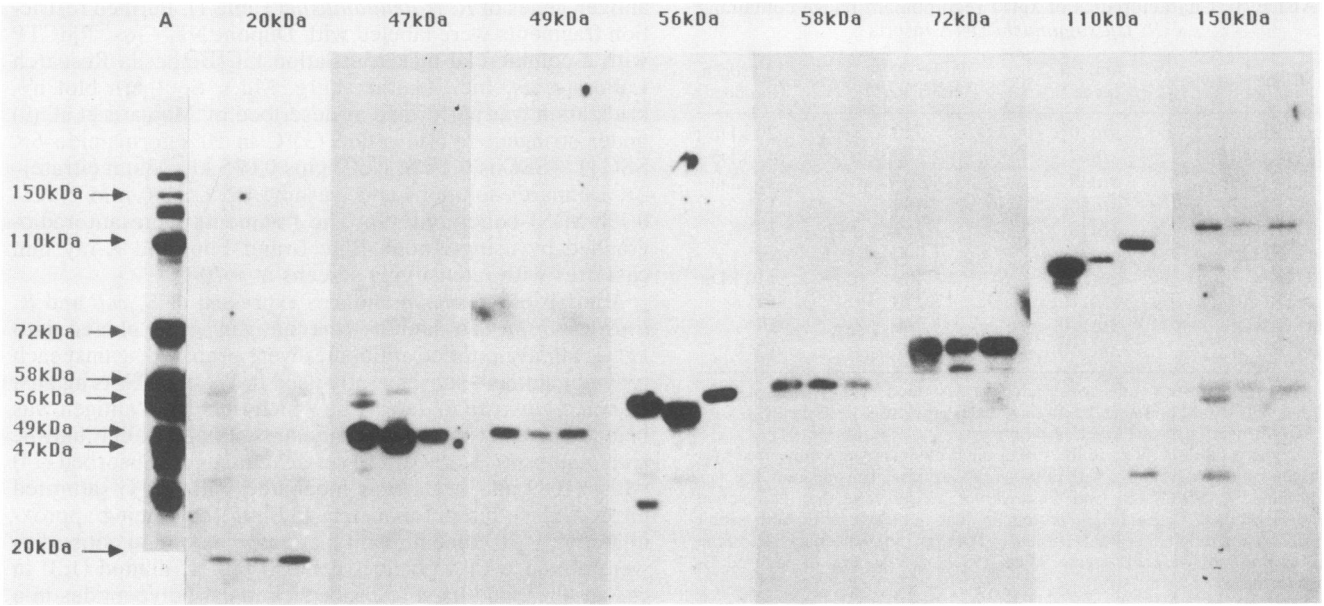

FIG. 1. Reactivity of recombinant antigen-selected antibodies with the Karp, Kato, and Gilliam strains of *R. tsutsugamushi*. Antibodies affinity purified against λgt11 recombinants expressing epitopes of different *R. tsutsugamushi* polypeptide antigens (indicated at the top of each panel) were incubated with electroblotted proteins of the Karp (left lane of each panel), Kato (middle lane), and Gilliam (right lane) strains. The strongest reacting rickettsial band for each affinity-purified antibody corresponds to the epitopes expressed by the recombinant. The weaker bands visible in some of the panels are most likely due to contaminating antibodies which copurified with the affinity purification. This is most notable for the 150-kDa antigen, which is a minor polypeptide and not readily detected by the rabbit sera. Long exposures are usually required for development of the 150-kDa antigen experiments, a condition which amplifies the background antibody reactivities. The apparent sizes of the Karp strain polypeptide antigens are indicated on the left side of the figure, next to lane A (the Karp strain antigens recognized by the hyperimmune rabbit serum).

eight different classes (Table 1) of antigen gene recombinant are described. These recombinants were used to affinity purify antibodies specific for each of the eight different protein antigens. In addition, cloned inserts from the λgt11 clones were used as DNA probes for the eight different antigen genes.

Antigenic relatedness of eight *R. tsutsugamushi* (strain Karp) polypeptides with the Kato and Gilliam strains. By using antibodies that had been affinity purified against recombinants expressing epitopes of the cloned genes, we were able to probe the Kato and Gilliam strains for cross-reactive proteins. Figure 1 shows a composite of several Western blots in which affinity-purified antibodies were reacted with SDS lysates of the Karp, Kato, and Gilliam strains. Cross-reactive epitopes for all eight Karp antigens were found in the Kato and Gilliam strains. As reported earlier (14), slight differences in the apparent polypeptide size was evident in the 110- and 56-kDa Karp strain antigens and the corresponding cross-reactive polypeptides in the Kato and Gilliam strains. All other polypeptide antigens had similar electrophoretic mobilities in the three reference *R. tsutsugamushi* strains.

Southern blot analysis of *R. tsutsugamushi* genomic DNA. Probes specific for DNA associated with the eight antigen genes were prepared by isolating cloned inserts from recombinant λgt11 molecules expressing determinants of the antigens and labeled with [^{32}P]ATP by nick translation. Genomic DNA of the *R. tsutsugamushi* Karp, Kato, and Gilliam strains was digested with *Sau*3A or *Taq*I restriction enzymes, resolved by agarose gel electrophoresis (1.2%), and subjected to Southern blot hybridization analysis with the antigen gene probes. Hybridization and washing were performed under stringent conditions for *R. tsutsugamushi* DNA, which is 65% AT rich (C. K. Stover and E. V. Oaks, unpublished data). A unique restriction fragment profile resulted for each of the eight antigen gene probes. Strong homology was observed between each of the Karp strain antigen gene probes and the genomic DNA restriction fragments of the Kato and Gilliam strains (Fig. 2). However, restriction fragment length polymorphisms were observed with every antigen gene probe in at least one digest and one strain of each hybridization. Restriction fragments associated with the *sta*20 and *sta*56 genes were the most polymorphic, while fragments associated with the *sta*49 and *sta*72 genes appeared to be the most conserved. Because each antigen gene probe may also carry DNA flanking the antigen genes, it is possible that some restriction fragment length polymorphisms are not due to strain-specific sequences in the structural genes of the antigens. This analysis did reveal that the Kato and Gilliam strains have DNA strongly homologous to that of the antigen genes of the Karp strain but that it is likely that there are strain-specific sequences in at least some antigen genes of *R. tsutsugamushi*. Hybridizations with a probe containing the entire *sta*110 gene and approximately 2 kilobase pairs (kb) of flanking DNA indicated that the *sta*110 gene, the flanking DNA, or both are repeated in the chromosomes of all three strains of *R. tsutsugamushi*, as the number and cumulative size of the fragments hybridizing to the 5.4-kb probe are too large to be explained by a single genomic copy.

Analysis of recent *R. tsutsugamushi* human isolates with affinity-purified antibodies. The reference Karp, Kato, and Gilliam strains have a long passage history in laboratory animals and tissue culture cells. To determine whether the epitopes expressed by the Karp recombinants are representative of scrub typhus rickettsiae isolated more recently,

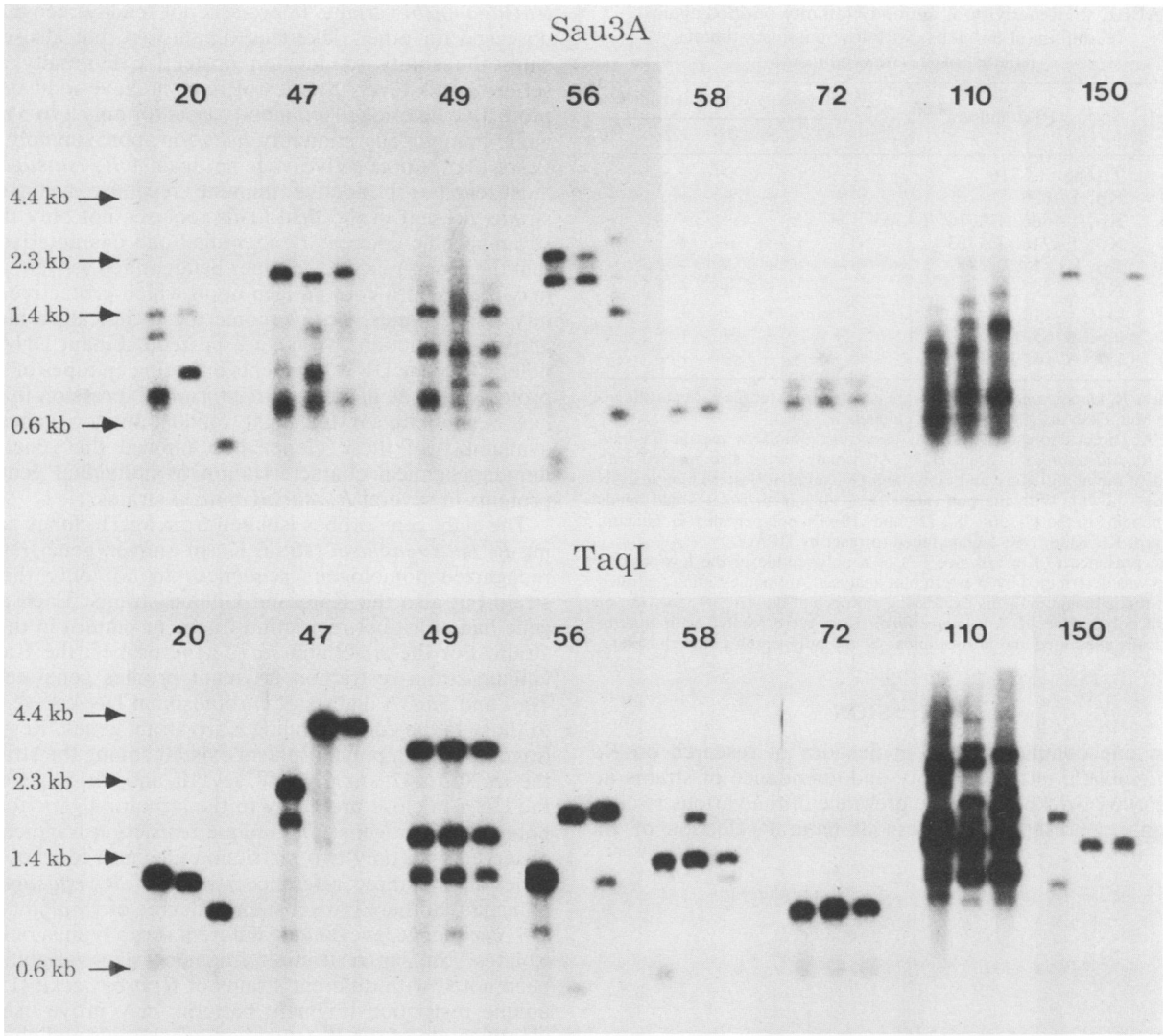

FIG. 2. Southern blot analysis of *R. tsutsugamushi* Karp, Kato, and Gilliam by using antigen gene probes isolated from λgt11 recombinant organisms expressing scrub typhus antigens. *R. tsutsugamushi* chromosomal DNA from the Karp, Kato, and Gilliam strains was digested with either *Sau*3A or *Taq*I restriction enzyme, subjected to agarose gel electrophoresis, and following transfer to nitrocellulose, probed with ^{32}P-labeled DNA probes obtained from recombinants expressing epitopes of the 20-, 47-, 49-, 56-, 58-, 72-, 110-, and 150-kDa polypeptide antigens. The three lanes in each probe column correspond to the Karp (left lane), Kato (middle lane), and Gilliam (right lane) strains. The *Sau*3A and *Sau*3A 58- and 150-kDa blots are weak but do contain reactive bands in all three strains. Molecular size markers are indicated on the left side of the figure.

several recent human isolates with low-passage histories were analyzed with the affinity-purified antibodies against the 47-, 56-, 58-, 72-, and 110-kDa polypeptide antigens.

Nine human *R. tsutsugamushi* isolates, previously determined to have a variety of antigenic compositions based on a direct fluorescent-antibody test (18), were obtained from hospitalized, febrile patients in the Mentekab district hospital in Pahang, Malaysia. Epitopes cross-reactive with the Karp 58-kDa polypeptide were found in all nine recent isolates, whereas epitopes cross-reactive with the 56-kDa (8 isolates), 72-kDa (8 isolates), and 47-kDa (8 isolates) polypeptides were found in most of the isolates (Table 2). Epitopes cross-reactive with the 110-kDa polypeptide were found in only four of nine isolates, and interestingly, all three isolates serotyped as Gilliam or Gilliam-like did not react with this affinity-purified antibody. These anti-110-kDa polypeptide affinity-purified antibodies used to probe the human isolates were prepared from λgt11Rts14, which expresses a complete 110-kDa polypeptide.

Strain-specific epitopes on the 110-kDa polypeptide. The inability of antibodies affinity purified against the recombinant 110-kDa polypeptide to react with several recent isolates serotyped as Gilliam or Gilliam-like suggests that the 110-kDa polypeptide most likely contains strain-specific epitopes like the 56-kDa polypeptide. Analysis of additional 110-kDa λgt11 recombinants, expressing portions of the 110-kDa polypeptide, identified one clone (λgt11Rts407) that made a fusion protein expressing a strain-specific region of the 110-kDa polypeptide. Figure 3 shows that antibodies affinity purified against λgt11Rts407 reacted predominantly with the Karp strain 110-kDa polypeptide, indicating that a strain-specific epitope exists in this polypeptide. However, on longer exposures it was possible to demonstrate weak reactivity with the Kato and Gilliam 110-kDa analogs.

TABLE 2. Reactivity of antibody (affinity purified against recombinant antigens) with low-passage human isolates of *R. tsutsugamushi*

Isolate[a]	DFA antisera[b]	Reactivity with polypeptide[c]				
		47	56	58	72	110
7506	TA686, TA716	−	+	+	−	−
6583	Kp, TA686	+	+	+	+	+
4716	Kp, TA686, TA716, TA763	+	+	+	+	+
4654	Kp, TA716, TA763	+	+	+	+	+
4673	Kp, Kt, TA716	+	+	+	+	−
7508	NR	+	+	+	+	+
7569	G	+	+	+	+	−
6802	G, TA763	+	+	+	+/−	−
7660	G, TA716, TA763	+/−	−	+	+/−	−

[a] These *R. tsutsugamushi* strains were isolated from febrile patients attending the Mentekab district hospital in Pahang.

[b] DFA, Direct fluorescent-antibody assay using antisera specific for individual *R. tsutsugamushi* strains (18). All isolates were also reacted with polyvalent rabbit anti-Karp and rabbit anti-Gilliam in a Western blot analysis (data not shown). With the polyvalent sera, all human isolates had bands corresponding to the 47-, 56-, 58-, 72-, and 110-kDa polypeptides. G, Gilliam; Kp, Karp; Kt, Kato. NR, Isolate failed to react by DFA.

[c] The presence (+) or absence (−) of a polypeptide in the low-passage isolates was determined by Western blot analysis. Antibodies affinity purified against recombinant antigens expressing epitopes of the 47-, 56-, 58-, 72-, or 110-kDa polypeptide of *R. tsutsugamushi* Karp were reacted with electrophoretically separated and nitrocellulose-bound polypeptides of each isolate.

DISCUSSION

The one common theme in decades of research on *R. tsutsugamushi* is the diversity and abundance of strains in this group of rickettsiae. The presence of the various strains not only represents a process of natural selection of *R. tsutsugamushi* variants (a process not readily recognized or observed for other rickettsial organisms), but also complicates the ability to develop protective immunity against scrub typhus fever. Studies of humans have indicated that protective heterlogous immunity lasts for only 1 to 3 months while homologous immunity lasts for approximately 1 to 3 years (21). An effective vaccine against *R. tsutsugamushi* must elicit a protective immune response against most strains present in the field. This requires not only that the strain-specific epitopes be identified and characterized, but that the group-reactive epitopes be identified as well, as they may represent a core antigen upon which protective immunity also depends. To overcome the technical problems of antigen production, we have used recombinant DNA technology to clone DNA fragments encoding epitopes of several protein antigens of *R. tsutsugamushi*. Expression in *E. coli* has been obtained for eight unique antigen genes. The availability of these clones has allowed the genetic and immunochemical characterization of individual genes and proteins in several *R. tsutsugamushi* strains.

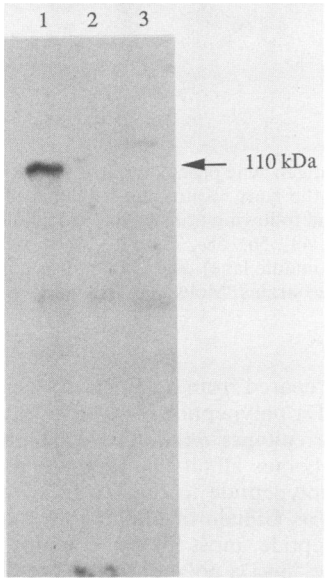

FIG. 3. Strain-specific reactivity of antibodies affinity purified with recombinant λgt11Rts407. Antibodies affinity purified with recombinant λgt11Rts407, which synthesizes a fusion protein containing a portion of the 110-kDa polypeptide of *R. tsutsugamushi* Karp, were reacted with the Karp (lane 1), Kato (lane 2), and Gilliam (lane 3) strains in a Western blot procedure. The bound antibodies were detected with iodinated protein A. The dominant reaction in this 24-h exposure autoradiograph was against the Karp 110-kDa polypeptide, as indicated by the arrow on the right side of the gel.

The eight gene probes isolated from λgt11 clones containing *R. tsutsugamushi* (strain Karp) antigen gene fragments recognized homologous sequences in not only the Karp strain but also the Kato and Gilliam strains. Each antigen gene had a distinct restriction fragment pattern in the Karp strain. For the *sta*49 and *sta*72 gene probes, the Kato and Gilliam strain restriction fragment profiles generated with *Taq*I and *Sau*3A digests of chromosomal DNA were similar to those in the corresponding Karp strain genes. Restriction fragment length polymorphism existed among the strains for the *sta*20, *sta*47, *sta*56, *sta*58, *sta*110, and *sta*150 genes or in the DNA in close proximity to the structural gene for these polypeptide antigens. The unique restriction fragment sizes observed with only two restriction enzymes for these latter genes in the three reference strains of *R. tsutsugamushi* indicate that there is a substantial degree of variability at the DNA sequence level among different scrub typhus rickettsial isolates. Similar restriction fragment size variability has been noted with different strains of *R. prowazekii* (15). The unique restriction fragment patterns may prove useful for identification of the *R. tsutsugamushi* strains and characterization of new isolates. This would be especially true if several gene probes were used. This type of analysis would also assist in the identification of mixed *R. tsutsugamushi* cultures, a problem that is thought to occur in rickettsiae isolated from chiggers and mammals (24).

The presence of homologous gene sequences among the three reference scrub typhus rickettsial strains was strong evidence that a corresponding antigen was also present. In both the Kato and Gilliam strains a polypeptide corresponding to each Karp polypeptide antigen was identified by using antibodies affinity purified against the recombinants expressing epitopes of the various Karp antigens. The cross-reactive polypeptide antigens had similar mobilities in SDS-PAGE gels, except for those antigens corresponding to the 110- and 56-kDa polypeptides of the Karp strain. Both of these polypeptides have electrophoretic mobilities that are unique to each strain.

By using affinity-purified antibodies against a λgt11 clone expressing only a portion of the 110-kDa polypeptide, it was possible to show that the 110-kDa polypeptide contains epitopes that are not strongly cross-reactive with the Kato (115-kDa) and Gilliam (130-kDa) polypeptides. This is the second *R. tsutsugamushi* polypeptide characterized as having strain-specific epitopes, the first being the 56-kDa polypeptide (7, 23). As mentioned above, it is interesting that

both the 56- and 110-kDa polypeptides and the corresponding cross-reactive antigens in the heterologous strains have distinctive mobilities in polyacrylamide gels. This suggests that both a common core structure (containing cross-reactive epitopes) and a unique structure (containing strain-specific epitopes) exist in both of these polypeptide antigen groups.

A survey of nine human *R. tsutsugamushi* isolates determined that many of the epitopes present in the Karp strain, which was isolated in 1943 (4), are also present in strains recently isolated. Epitopes crossreactive with the 58-, 72-, 56-, and 47-kDa polypeptide antigens of the Karp strain were found most frequently in the low-passage isolates. Only epitopes of the 58-kDa antigen were found in all human *R. tsutsugamushi* isolates. Interestingly, recent analysis (C. K. Stover, E. V. Oaks, S. L. Pyle, and B. A. Roe, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, D64, p. 93) of the 58-kDa antigen gene sequence indicates that it is highly homologous with the heat shock family of proteins found in other bacteria, including *Mycobacterium tuberculosis* (17) and *Coxiella burnetii* (25). Out of four isolates which contained a polypeptide analogous to the Karp 110-kDa antigen, three were serotyped by DFA as Karp or Karp-like. The fourth isolate (7508) expressing a 110-kDa polypeptide analog was not serotyped by DFA, but it behaved like a Karp strain in that it reacted with all the antibodies that had been affinity purified against the recombinant antigens. Three of the five strains which were not reactive with the antibodies affinity purified against the recombinant (λgt11Rts14) 110-kDa polypeptide were Gilliam or Gilliam-like. Previously, we have shown similar weak results with the Gilliam strain with antibodies purified against clone λgt11Rts14 (14). The lack of detection of a particular polypeptide with antibodies affinity purified with recombinant antigens does not rule out the presence of a similar polypeptide expressing different epitopes. In fact when the low-passage human isolates were reacted with anti-Gilliam hyperimmune rabbit sera, all of the isolates exhibited a band in the 110- to 130-kDa region (data not shown).

This study has clearly shown that eight different antigen genes and their polypeptide antigen products of *R. tsutsugamushi* Karp share conserved DNA sequences and epitopes with similar genes and products in the Kato and Gilliam strains. The presence of strain-specific epitopes on different *R. tsutsugamushi* polypeptides indicates that the variation between strains may represent a mosaic of antigenic structures and that it will be difficult to determine which epitopes are responsible for homologous immunity. At this point, it is not clear what the mechanism of antigenic diversity is, i.e., it could be due to the presence of multiple gene copies (possibly in the case of the *sta*110 gene) encoding different epitopes similar to those of the pilin genes of *Neisseria gonorrhoeae* (16), or the antigen genes may undergo mutations giving rise to new strains or epitopes. A lipopolysaccharidelike structure may also be involved in antigenic diversity of *R. tsutsugamushi*, as in many gram-negative enteric bacteria (for example, *Salmonella* spp.), but because a lipopolysaccharidelike molecule has not been detected in *R. tsutsugamushi* (1) this type of antigenic variation may not be possible.

With the strong DNA sequence homology, it will be possible to use the Karp strain antigen gene recombinants as probes to clone and eventually sequence antigen genes of other *R. tsutsugamushi* strains. The determination of strain-specific nucleotide sequences may provide answers to epidemiological questions posed years ago by Shirai and Wisseman (19) about the origin and development of *R. tsutsugamushi* antigenic diversity.

LITERATURE CITED

1. **Amano, K., A. Tamura, N. Ohashi, H. Urakami, S. Kaya, and K. Fukushi.** 1987. Deficiency of peptidoglycan and lipopolysaccharide components in *Rickettsia tsutsugamushi*. Infect. Immun. **55**:2290–2292.
2. **Bozeman, F. M., and B. L. Elisberg.** 1963. Serological diagnosis of scrub typhus by indirect immunofluorescence. Proc. Soc. Exp. Biol. Med. **112**:568–573.
3. **Brown, G. W., D. M. Robinson, D. L. Huxsoll, T. S. Ng, K. J. Lim, and G. Sannasey.** 1976. Scrub typhus: a common cause of illness in indigenous populations. Trans. R. Soc. Trop. Med. Hyg. **70**:444–448.
4. **Derrick, E. H., and H. E. Brown.** 1949. Isolation of the Karp strain of *Rickettsia tsutsugamushi*. Lancet **ii**:150–151.
5. **Eisemann, C. S., and J. V. Osterman.** 1985. Identification of strain-specific and group-reactive antigenic determinants on the Karp, Gilliam and Kato strains of *Rickettsia tsutsugamushi*. Am. J. Trop. Med. Hyg. **34**:1173–1178.
6. **Elisberg, B. L., J. M. Campbell, and F. M. Bozeman.** 1968. Antigenic diversity of *Rickettsia tsutsugamushi*: epidemiologic and ecologic significance. J. Hyg. Epidemiol. Microbiol. Immunol. **12**:18–25.
7. **Hanson, B.** 1986. Identification and partial characterization of *Rickettsia tsutsugamushi* major protein immunogens. Infect. Immun. **50**:603–609.
8. **Hunter, W. M., and F. C. Greenwood.** 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) **194**:495–496.
9. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Myers, W. F., and C. L. Wisseman, Jr.** 1980. Genetic relatedness among the typhus group of rickettsiae. Int. J. Syst. Bacteriol. **30**:143–150.
11. **Myers, W. F., and C. L. Wisseman, Jr.** 1981. The taxonomic relationship of *Rickettsia canada* to the typhus and spotted fever groups of the genus *Rickettsia*, p. 313–325. *In* W. Burgdorfer and R. L. Anacker (ed.), Rickettsiae and rickettsial diseases. Academic Press, Inc., New York.
12. **Myers, W. F., C. L. Wisseman, Jr., P. Fiset, E. V. Oaks, and J. F. Smith.** 1979. Taxonomic relationship of Vole agent to *Rochalimaea quintana*. Infect. Immun. **26**:976–983.
13. **Oaks, E. V., T. L. Hale, and S. B. Formal.** 1986. Serum immune response to *Shigella* protein antigens in rhesus monkeys and humans infected with *Shigella* spp. Infect. Immun. **53**:57–63.
14. **Oaks, E. V., C. K. Stover, and R. M. Rice.** 1987. Molecular cloning and expression of *Rickettsia tsutsugamushi* genes for two major protein antigens in *Escherichia coli*. Infect. Immun. **55**:1156–1162.
15. **Regnery, R. L., T. Tzianabos, J. J. Esposito, and J. E. McDade.** 1983. Strain differentiation of epidemic typhus rickettsiae (*Rickettsia prowazekii*) by DNA restriction endonuclease analysis. Curr. Microbiol. **8**:355–358.
16. **Segal, E., P. Hagblom, H. S. Seifert, and M. So.** 1986. Antigenic variation of gonococcal pilus involves assembly of separated silent gene segments. Proc. Natl. Acad. Sci. USA **83**:2177–2181.
17. **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.
18. **Shirai, A., D. M. Robinson, G. W. Brown, E. Gan, and D. L. Huxsoll.** 1979. Antigenic analysis by direct immunofluorescence of 114 isolates of *Rickettsia tsutsugamushi* recovered from febrile patients in rural Malaysia. Jpn. J. Med. Sci. Biol. **32**:337–344.
19. **Shirai, A., and C. L. Wisseman, Jr.** 1975. Serological classification of scrub typhus isolates from Pakistan. Am. J. Trop. Med. Hyg. **24**:145–153.
20. **Sidberry, H., B. Kaufman, D. C. Wright, and J. Sadoff.** 1985. Immunoenzymatic analysis by monoclonal antibodies of bacte-

rial lipopolysaccharides after transfer to nitrocellulose. J. Immunol. Methods **76**:299–305.

21. **Smadel, J. E., H. L. Ley, F. H. Diercks, P. Y. Paterson, C. L. Wisseman, Jr., and R. Traub.** 1952. Immunization against scrub typhus: duration of immunity in volunteers following combined living vaccine and chemoprophylaxis. Am. J. Trop. Med. Hyg. **1**:87–99.
22. **Stover, C. K., M. H. Vodkin, and E. V. Oaks.** 1987. Use of conversion adaptors to clone antigen genes in lambda gt11. Anal. Biochem. **163**:398–407.
23. **Tamura, A., N. Ohashi, H. Urakami, K. Takahashi, and M. Oyanagi.** 1985. Analysis of polypeptide composition and antigenic components of *Rickettsia tsutsugamushi* by polyacrylamide gel electrophoresis and immunoblotting. Infect. Immun. **48**:671–675.
24. **Traub, R., and C. L. Wisseman, Jr.** 1974. The ecology of chigger-borne rickettsiosis (scrub typhus). J. Med. Entomol. **11**:237–303.
25. **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.
26. **Weiss, E., and J. W. Moulder.** 1984. The *Rickettsias* and *Chlamydias*, p. 687–739. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
27. **Yamashita, T., S. Kasuya, S. Noda, I. Nagano, S. Ohtsuka, and H. Ohtomo.** 1988. Newly isolated strains of *Rickettsia tsutsugamushi* in Japan identified by using monoclonal antibodies to Karp, Gilliam, and Kato strains. J. Clin. Microbiol. **26**:1859–1860.
28. **Young, R. A., and R. W. Davis.** 1983. Yeast RNA polymerase II genes: isolation with antibody probes. Science **222**:778–782.