Comparative Virulence of Intra- and Interstrain Lipopolysaccharide Variants of *Coxiella burnetii* in the Guinea Pig Model

ABBIE MOOS AND TED HACKSTADT*

Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840

Received 14 October 1986/Accepted 21 January 1987

We compared the relative infectivity and virulence of lipopolysaccharide (LPS) variants of the Nine Mile strain of *Coxiella burnetii* with those of the Priscilla strain, a representative of endocarditis-type strains. In agreement with results of previous studies, Nine Mile phase I (9mi/I) organisms were highly infectious, eliciting seroconversion and fever with inocula containing as few as four organisms. Viable 9mi/I was recovered from the spleens of infected animals 30 days postinfection. Nine Mile phase II (9mi/II) organisms did not elicit fever or seroconversion except with very large inocula, and viable organisms could not be recovered at 30 days postinfection. The Nine Mile/Crazy variant, bearing the intermediate-type LPS, was also highly infectious, as determined by fever response and seroconversion, although, as with 9mi/II, viable organisms could not be recovered 30 days postinfection. The Priscilla strain in phase I (Pris/I) was as infectious as 9mi/I, as determined by seroconversion and its presence in the spleen 30 days postinfection; but in contrast to 9mi/I, more than 10^5 Pris/I isolates were required to induce fever. The temporal appearances of anti-phase I and II antibodies were similar for the two strains. A variety of serological techniques measuring antibody response against whole-cell and purified LPS antigens in agglutination, immunofluorescence, enzyme-linked immunosorbent, and immunoblot assays did not demonstrate sufficient specificity to distinguish between 9mi/I and Pris/I infections. Results of vaccine cross-challenge experiments showed a significant degree of protection between homologous and heterologous challenge strains.

* C. burnetii, the etiologic agent of Q fever, is unique among members of the family *Rickettsiacea* in that it undergoes lipopolysaccharide (LPS) phase variation. This phase variation parallels the smooth to rough LPS variation of gram-negative enteric bacteria (1, 12, 36). Virulent phase I *C. burnetii*, isolated from natural infections, has a more complex smooth-type LPS. Avirulent phase II *C. burnetii* has a rough-type LPS and is selected for during laboratory passage in nonimmunologically competent hosts such as eggs or tissue culture (3). In addition, a phenotypic mutant bearing an LPS that is structurally intermediate between phase I and phase II has recently been characterized, Nine Mile/Crazy (9mi/Cr) (12). The correlation of LPS structure with virulence suggests a role for LPS in the pathogenesis of this organism.

By using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting techniques, three groups of phase I isolates of *C. burnetii* have recently been established based on antigenic or structural variation of their LPSs (11). Group I has an LPS profile similar to that of the well-characterized Nine Mile phase I (9mi/I) strain. All isolates in this group are from patients with primary (acute) Q fever, ticks, or milk from various geographic locations. The other two groups are composed of isolates from patients with chronic Q-fever endocarditis and include a single isolate from a goat placenta (Priscilla strain [Pris]). Based on LPS profile, group 2, which comprises Pris, P, K, and F isolates, appears to be most distinct from group 1. Purified LPSs from this group showed only weak cross-reactivity with rabbit antiserum prepared against 9mi/I. The converse was also true because antiserum prepared against Pris in Phase I (Pris/I), which is typical of group 2, reacted primarily with LPSs of that class. Group 3 consists of S, Ko, G, and L isolates; although this group has a unique LPS migration pattern, it is antigenically related to group 1 (11). Restriction endonuclease mapping of *C. burnetii* plasmids has demonstrated an association between plasmid type and disease entity (33), and the groupings based on plasmid profile correlate precisely with those based on LPS migration pattern.

Having identified groups that correlate with isolation from different disease processes and that differ in a prominent surface antigen, we used the guinea pig model to examine the biological activity of two strains that demonstrated structural and antigenic differences. We compared the 9mi/I strain (acute Q-fever group 1) with the Pris/I strain (chronic Q-fever group 2). Purified LPSs from groups 1 and 2 were antigenically distinct and showed the greatest degree of structural variation. In this study we focused primarily on virulence properties of these two strains, serological responses toward these different strains, pyrogenic effects of purified LPSs, and protection against homologous and heterologous challenge following vaccination with whole Formalin-killed *C. burnetii* organisms.

**MATERIALS AND METHODS**

**Organisms.** Strain histories, propagation (11, 12), and purification procedures (40) have been described previously.

**Enumeration techniques.** (i) Direct particle count. Serial dilutions of Formalin-killed *C. burnetii* organisms (seed stock concentration, 1 mg [dry weight]/ml) were stained with 0.01% (final concentration) acridine orange (16). The samples were then filtered under vacuum through Nuclepore filters (Nucleopore Corp., Pleasanton, Calif.) that were previously stained with 0.02% (wt/vol) irgamar black in 2% (vol/vol) acetic acid (16, 31) to reduce autofluorescence. A standard epifluorescence microscope (Zeiss) was used. The numbers of bacteria per milliliter were estimated from the
average count of at least 15 randomly chosen microscopic fields.

(ii) Inclusion-forming units. The technique described by Hahon and Cooke (14) was used for the inclusion-forming unit(s) (IFU) assay on Vero cell monolayers.

(iii) PFU. PFU on chicken embryo fibroblasts were determined as described by Ormsbee and Peacock (27).

Pyrogen test. The pyrogenicity of purified Coxiella LPS was tested in New Zealand White rabbits (weight, 1.5 to 2 kg) essentially as described by Keene et al. (20). Increasing doses of purified Coxiella LPS (0.25 to 100 μg per animal) in endotoxin-free water (Sigma Chemical Co., St. Louis, Mo.) were injected intravenously into rabbits that were previously acclimatized to holding stocks and a rectal probe. Rectal temperatures were recorded every 15 min for 5 h. The fever curve was compared with that produced by a reference endotoxin (Escherichia coli O111 B4) (Sigma) and a diluent control.

Serologic assays. (i) Microagglutination assay. Phase I whole-cell antigens (9mi/I and Pris/I) and Nine Mile phase II (9mi/II) antigen were prepared as described by Fiset et al. (10). The microagglutination (MA) assay was also performed as described previously (10).

(ii) Enzyme-linked immunosorbent assay. The procedure described by Field et al. (8) was used for the enzyme-linked immunosorbent assay (ELISA), but with the modifications. LPS purified by the methods described above was used as the test antigen. U-bottomed micro-ELISA plates (Immulon-2; Dynatech Laboratories, Inc., Alexandria, Va.) were coated with 50 μg of the test antigen (2.5 μg of purified LPS per ml from 9mi/I or Pris/I organisms) in 0.06 M carbonate buffer (pH 9.5) and incubated at 37°C for 3 h. The plates were then stored at 4°C until used. Following three washes with phosphate-buffered saline (PBS; pH 7.2) containing 0.05% (vol/vol) Tween 20 (PBS-T), 50-μl fractions of serially diluted sera in PBS-T were added. The plates were incubated for 1 h at 37°C. After three washes with PBS-T, 50 μl of a 1:2,000 dilution of rabbit anti-guinea pig immunoglobulin G horseradish peroxidase conjugate (Miles Laboratories, Inc., Elkhart, Ind.) was added. The plates were incubated at 37°C for 1 h and washed three times with PBS-T, then 50 μl of substrate (O-phenylendiamine dihydrochloride) was added. The plates were left in the dark for 30 min. The reaction was stopped by the addition of 25 μl of 4 M H2SO4. Optical density readings were determined at 492 nm in an ELISA reader (Titertec Multiskan) and values greater than or equal to 0.20 were recorded as positive.

(iii) Microimmunofluorescence assay. The microimmunofluorescence (MIF) assay was essentially as described by Philip et al. (32). Fluorescein isothiocyanate-conjugated rabbit anti-guinea pig conjugate (Cappel Laboratories, West Chester, Pa.) was used. The endpoint was the highest serum dilution conferring definite fluorescence to the antigen in question.

Vaccination and challenge experiment. Female Hartley guinea pigs (age, 6 to 8 weeks) were divided into three groups. One group was vaccinated intramuscularly (i.m.) with 20 μg (dry weight) of Formalin-killed 9mi/I organisms, the second group was vaccinated with 20 μg of Formalin-killed Pris/I organisms, and the third group served as a control group and was given i.m. injections of isotonic saline. Thirty days after vaccination, each group was subdivided into three groups and challenged intraperitoneally (i.p.) with 107 organisms of 9mi/I or Pris/I diluted in beef heart infusion broth (BHI) or a control injection of BHI. Rectal temperatures were recorded in all animals for 10 days.

FIG. 1. Silver-stained SDS-PAGE profile of purified Pris/I (Pr/I) and 9mi/I LPSs and whole-cell lysates (A). (B) An autoradiogram of lactoperoxidase-catalyzed surface iodinated Pris/I and 9mi/I. Molecular weight markers (102) are indicated between the panels.

The animals were anesthetized (6.25 mg of ketamine and 1.25 mg of xylazine kg−1 body weight) and bled by cardiac puncture on days 0 and 60 (30 days after challenge). MA titers to 9mi/I, 9mi/II, and Pris/I whole-cell antigens were measured. One week later, the guinea pigs were euthanized, and the spleens were removed aseptically and homogenized to a 50% (wt/vol) suspension with BHI. A 0.2-ml fraction of spleen suspension was injected i.p. into white mice (Rocky Mountain Laboratories), with two mice receiving injections from a single spleen. Thirty days following injection, the mice were bled from the tail artery and MA titers were measured.

SDS-PAGE, immunoblotting, and surface iodination. Procedures for SDS-PAGE and immunoblotting have been described previously (13). Silver staining was done by the method of Tsai and Frasch (38). Surface iodination was done by the lactoperoxidase procedure (25).

LPS isolation. LPS was extracted from purified C. burnetii organisms by the hot phenol-water method described by Westphal and Jann (39), as modified by Hackstadt et al. (12).

RESULTS

Comparison of Pris/I and 9mi/I by SDS-PAGE. The SDS-PAGE profile of whole-cell lysates and phenol-water-extracted LPS of Pris/I and 9mi/I is depicted in Fig. 1. As described previously (11), the LPSs of these two strains differ in their electrophoretic mobilities, as assessed by silver-stained SDS-PAGE, and antigenically, as assessed by immunoblotting with rabbit hyperimmune antisera. The most obvious differences in the SDS-PAGE profile of purified LPS are in the region of the 14.3-kilodalton marker (11). Polypeptide profiles in whole-cell lysates were strikingly similar,
with minor differences apparent in the 14- to 25-kilodalton range. However, this region of the gel is the area of phase I LPS migration, and the peptide banding patterns in this region may be obscured by comigration with phase I LPS (12). An analysis of surface-exposed proteins was made by comparing proteins labeled by lactoperoxidase-catalyzed radiodination (Fig. 1). The most prominent surface-labeled proteins were shared between Pris/I and 9mi/II; however, some differences were detected at about 43 and 14 to 25 kilodaltons.

Enumeration and comparative infectivity of *C. burnetii* strains. Comparison of relative virulence required the accurate enumeration of total and viable organisms in the inoculum (Table 1). The numbers of infectious organisms of Pris/I, 9mi/I, 9mi/Cr, and 9mi/II were assayed by several techniques. Direct particle counts of 9mi/I, 9mi/Cr, and Pris/I on black Nucleopore filters gave equivalent values (3.4 \times 10^{10} to 5.3 \times 10^{10} cells per ml). A slightly higher value was observed for the 9mi/II stock. Numbers of viable organisms were determined by assay of PFU on chicken embryo fibroblasts, IFU on Vero cells, and infectivity in guinea pigs.

The most sensitive assay for the detection of viable organisms was infectivity in guinea pigs. Seroconversion was observed following inoculation with as few as two to four organisms per animal of either 9mi/I or Pris/I. The numbers of IFU on Vero cells were comparable for 9mi/I (1.8 \times 10^6 IFU/ml) and Pris/I (1.6 \times 10^6 IFU/ml), but these values underestimate the true number of viable organisms based on infectivity in the guinea pig. Plaque formation could not be used for quantitating viable organisms because the Pris/I isolate did not form plaques on chick embryo fibroblasts, and the number of plaques formed by the 9mi/I isolate (3.4 \times 10^7 plaques per ml) again underestimated the number of viable organisms in the seed stock.

Determination of numbers of viable 9mi/II organisms presented more of a problem because 9mi/II is avirulent and we could not titrate its infectivity in animals, which was the most sensitive method for phase I organisms. IFUs on Vero cells and PFU on chick embryo fibroblasts were greater for both 9mi/II and 9mi/Cr than for 9mi/I, yet we could not directly demonstrate that viability of 9mi/II was equal to that of 9mi/I or Pris/I, in which the particle-to-infectivity ratio approached 2:1.

Fever response to challenge with LPS variants. LPS variants within the Nine Mile strain were compared in their ability to elicit fever in guinea pigs (Fig. 2). Phase II organisms caused no fever except at the highest dose (10^8 organisms), and even at this dose, fever lasted no longer than 3 days in any single animal. In contrast, challenge with 9mi/Cr organisms produced a febrile response comparable to that of the 9mi/I challenge (although 9mi/Cr was not tested at doses of less than 100 organisms). Four of five animals inoculated with 10 9mi/I organisms and five of six animals inoculated with 10^2 9mi/I developed fever at some time during the experiment. All animals receiving larger doses of 9mi/I developed fever. Five of six animals receiving 10^2 9mi/Cr developed fever, but only two of six guinea pigs receiving 10^4 9mi/Cr developed fever. The remainder of the animals inoculated with the higher doses of 9mi/Cr developed fever. All but one animal inoculated with 10 or more 9mi/I organisms or 10^2 or more 9mi/Cr organisms showed evidence of infection, as assessed by seroconversion, whether or not they displayed fever. The 9mi/Cr-infected animals developed agglutinating antibodies to phase II antigen only (data not shown). This differed from the results of 9mi/I infection, during which agglutinating antibodies to both phase I and II antigens were produced (see below). An important feature that distinguished phase I infection from infection with the organisms possessing a more truncated LPS was that infectious microorganisms were isolated from the spleen 30 days after phase I challenge with 10^6 organisms (four of four animals), while no infectious organisms were recovered from animals challenged with the same dose of 9mi/Cr or 9mi/II.

Daily rectal temperatures of guinea pigs inoculated with various doses of Pris/I were also measured for 3 weeks (Fig.

### TABLE 1. Comparative infectivity and virulence of *C. burnetii* strains and phase variants

<table>
<thead>
<tr>
<th>Strain/phase</th>
<th>Particles/ml (10^10)</th>
<th>PFU/ml</th>
<th>IFU/ml</th>
<th>GPID&lt;sub&gt;90&lt;/sub&gt;</th>
<th>GPFD&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>9mi/I</td>
<td>3.4</td>
<td>3.4 \times 10^7</td>
<td>1.8 \times 10^6</td>
<td>4.2</td>
<td>4.8</td>
</tr>
<tr>
<td>9mi/Cr</td>
<td>3.5</td>
<td>6.8 \times 10^8</td>
<td>3.7 \times 10^7</td>
<td>&lt;10^2</td>
<td>&lt;10^2</td>
</tr>
<tr>
<td>9mi/II</td>
<td>4.7</td>
<td>7.9 \times 10^8</td>
<td>9.4 \times 10^6</td>
<td>10^2</td>
<td>10^7</td>
</tr>
<tr>
<td>Pris/I</td>
<td>3.6</td>
<td>1.7 \times 10^9</td>
<td>2.5</td>
<td>10^{-3}</td>
<td></td>
</tr>
</tbody>
</table>

\* Direct particle counts on black nucleopore filters.
\* PFU on chick embryo fibroblasts.
\* IFUs on Vero cells.
\* GPID<sub>90</sub>, Guinea pig infectious dose; the number of infectious organisms that elicit seroconversion in 50% of the animals.
\* GPFD<sub>90</sub>, Guinea pig fever dose; the number of infectious organisms that elicit a fever of \(\geq 40^\circ C\) in 50% of animals.
2. Only guinea pigs given $10^6$ or more Pris/I organisms developed fever. Animals inoculated with less than $10^6$ organisms did not develop fever, although like the 9mi/I- and 9mi/Cr-infected guinea pigs, they did show evidence of infection, as assessed by seroconversion (see below).

**Serologic response to experimental infection with 9mi/I or Pris/I.** Although Pris/I infection often did not result in fever, serologic responses of the guinea pigs to infection with $10^6$ organisms of either 9mi/I or Pris/I were very similar (Fig. 3). MA titers to Pris/I, 9mi/I, and 9mi/I antibodies were measured 7, 14, 21, and 30 days following infection. Both 9mi/I and Pris/I infections resulted in high phase II antibody titers at 14 days. Pris/I and 9mi/I antibodies were produced by 21 days. However, there was no apparent strain specificity in the serologic response of the guinea pigs (Table 2). While the 9mi/I-infected guinea pigs consistently had higher phase II titers than Pris/I-infected animals, no differences were apparent in the MA titers against either phase I antigen. The MIF assay and ELISA also failed to demonstrate sufficient specificity to distinguish between isolates. While the geometric mean titers directed against the homologous LPS antigen were slightly higher than those directed against the heterologous antigen, there was considerable overlap in individual values; thus, we do not consider these differences to be significant.

**Immunoblot analysis of whole-cell lysates of 9mi/I, Pris/I, 9mi/II, and 9mi/Cr with guinea pig sera from animals infected with either 9mi/I or Pris/I organisms.** Revealed multiple cross-reacting bands, representing protein and LPS epitopes common to both strains and the intrainstrain variants of Nine Mile (Fig. 4). Guinea pigs showed considerable individual variation in their response to C. burnetii proteins, regardless of the infecting strain. However, all immunoreactive proteins were shared by both phase I organisms and their LPS mutants. No differences in response to the extracted LPSs were observed, and the reactivities of antisera from 9mi/I- and Pris/I-infected guinea pigs were similar when blotted against homologous or heterologous LPS.

**Pyrogenicity of 9mi/I and Pris/I LPS in rabbits.** We compared the pyrogenicities of the purified 9mi/I and Pris/I LPSs in rabbits. The fever curves were compared with those of control rabbits receiving *E. coli* O111 B4 LPS. As has been described for 9mi/I LPS (2), neither 9mi/I nor Pris/I LPS induced a pyrogenic response even at doses as high as 100 μg. This is in contrast to *E. coli* LPS, which induced fever at doses of 0.15 μg (data not shown).

**Vaccination and challenge study.** In cross-protection experiments, guinea pigs were vaccinated with Formalin-killed whole organisms and challenged i.p. 30 days later with $10^5$ viable homologous or heterologous organisms. Rectal temperatures were measured for 10 days following challenge (Table 3). None of the vaccinated animals developed a fever. Both the 9mi/I and the Pris/I vaccines protected against fever in the guinea pigs challenged with 9mi/I organisms. The guinea pigs challenged with Pris/I organisms did not develop a fever, but neither did the unvaccinated (saline control) animals challenged with Pris/I, because the dose of Pris/I used does not typically induce fever.

| TABLE 2. Serologic titers of guinea pigs 30 days after infection with C. burnetii |
|------------------------|-----------------|-----------------|-----------------|-----------------|
| Infected strain*       | MA (range)      | MIF (range)     | ELISA (range)   |
|                        | I (10^5 Organisms) | I (10^5 Organisms) | I (10^5 Organisms) |
| 9mi/I                  | 15 (8–32)       | 512 (512)       | 512 (128–8,192) |
| Pris/I                 | 10 (8–16)       | 128 (128)       | 1,024 (256–4,096) |

* Animals were infected i.p. with $10^5$ organisms.

b Geometric mean titer; n = 4.

**FIG. 3.** Temporal appearance of phase I and II antibodies during experimental infection of guinea pigs with $10^5$ 9mi/I or Pris/I organisms. Antibody titers were determined by the MA technique (10).

**FIG. 4.** Representative immunoblot analysis of guinea pig antibody responses to various antigens after experimental infection with either 9mi/I or Pris/I (Pr/I). The lanes contain purified Pris/I or 9mi/I LPSs or whole-cell lysates (WC) or Pris/I, 9mi/I, 9mi/Cr, or 9mi/II. The response to 9mi/II whole-cell lysates may be taken as a response to proteinaceous antigens because this cell type lacks the phase I LPS antigen (12). Molecular weight markers (10^3) are indicated to the right of the gels.
TABLE 3. Protection of guinea pigs after vaccination and challenge with homologous and heterologous C. burnetii strains

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Challenge</th>
<th>No. of guinea pigs with fever/total</th>
<th>MA titer (range) 30 days postinfection</th>
<th>Recovery of C. burnetii 30 days postinfection/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>9mi/I</td>
<td>9mi/I</td>
<td>0/4</td>
<td>192 (128–256) 1,280 (1,024–2,048) 128 (64–256)</td>
<td>0/4</td>
</tr>
<tr>
<td>Pris/I</td>
<td>0/4</td>
<td></td>
<td>64</td>
<td>56 (32–64)</td>
</tr>
<tr>
<td>BHI</td>
<td>0/4</td>
<td></td>
<td>18 (8–32)</td>
<td>18 (8–16)</td>
</tr>
<tr>
<td>Pris/I</td>
<td>9mi/I</td>
<td>0/4</td>
<td>112 (64–128) 2,048 144 (64–256)</td>
<td>1/4</td>
</tr>
<tr>
<td>Pris/I</td>
<td>0/4</td>
<td></td>
<td>64 (32–128) 160 (128–256) 64 (32–128)</td>
<td>0/4</td>
</tr>
<tr>
<td>BHI</td>
<td>0/4</td>
<td></td>
<td>24 (16–32) 32 20 (16–32)</td>
<td>0/4</td>
</tr>
<tr>
<td>Saline</td>
<td>9mi/I</td>
<td>4/4</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>Pris/I</td>
<td>0/4</td>
<td></td>
<td>37 (16–64) 299 (128–512) 43 (32–64)</td>
<td>4/4</td>
</tr>
<tr>
<td>BHI</td>
<td>0/4</td>
<td></td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

- Guinea pigs were vaccinated i.m. with 20 μg of Formalin-killed 9mi/I or Pris/I organisms.
- Rectal temperatures were measured daily for 10 days following challenge.
- Geometric mean MA titers to 9mi/I, 9mi/II, and Pris/I antigens measured 30 days following challenge.
- Determined by seroconversion (MA) in mice 30 days after inoculation with guinea pig splenic homogenate. Values are numbers of guinea pigs from which viable organisms were recovered/total number of animals in the group.
- Number of animals developing a fever 10 days following challenge/total number of animals in the group.

MA titers to 9mi/I, 9mi/II, and Pris/I antigens 30 days following challenge are shown in Table 3. Vaccination titers (60 days after vaccination) without challenge (BHI control) were comparable in animals given either Pris/I or 9mi/I vaccine. Following challenge with either isolate, MA titers decreased. The highest titers resulted form 9 mi/I challenge, regardless of the vaccination strain.

To evaluate the protective efficacy of these vaccines, the spleens of the vaccinated, challenged guinea pigs were assayed for the presence of viable organisms. Thirty days after challenge, guinea pigs were inoculated i.p. with the presence of viable organisms in the guinea pig spleen was detected by seroconversion in the mouse. MA titers were measured daily for 10 days following inoculation. The sensitivity of the mice to challenge with either strain was equivalent to that of guinea pigs (unpublished data). None of the mice inoculated with spleen suspensions from guinea pigs vaccinated with the 9mi/I vaccine showed seroconversion. Two of the eight mice that were inoculated with spleen suspensions from guinea pigs vaccinated with the 9mi/I vaccine showed seroconversion. Both of these mice were inoculated with a spleen suspension from the same guinea pig. This guinea pig had phase I and II MA titers of 128 and 4,096, respectively, 30 days following challenge and did not develop fever. All mice that were inoculated with splenic suspensions from unvaccinated C. burnetii-challenged guinea pigs showed seroconversion, regardless of the challenge strain.

DISCUSSION

Until recently, C. burnetii was considered a unique species with little or no antigenic variation among strains from widely separated geographical sources. It has become apparent, however, from genetic analysis (33; L. P. Mallavia, personal communication), as well as structural and antigenic analyses of the phase I LPSs (11), that strain variation does indeed occur. The strains that differ from the better characterized laboratory strains (i.e., Nine Mile and Ohio) were, with a single exception, isolated from human cases of chronic Q-fever endocarditis. We sought to characterize the biological activities of strains that vary in a prominent surface antigen, the LPS, for several reasons. The association of LPS structure with phase variation and decreased virulence in organisms bearing a more truncated LPS (phase II) suggests that LPS may play a role in pathogenesis (18). In addition, phase I vaccines are 100 to 300 times more efficacious than phase II vaccines (26). This has been interpreted as evidence that phase I antigen (LPS) is the protective immunogen (24). Finally, our findings indicate that the LPSs are the major structural components that differ among C. burnetii isolates (11, 12).

The guinea pig has been used extensively as an experimental model for C. burnetii infections (9, 17, 21, 22, 30) and is still the model system of choice for the initial evaluation of Q-fever vaccines. The pathogenesis of the Nine Mile strain has been examined in some detail (15). In response to i.p. injection of live organisms, the guinea pig develops fever, with the magnitude, duration, and latency being dose dependent. Our results comparing virulence of phase variants within the Nine Mile strain are in agreement with those from previous studies (18, 28), in that the phase II organisms are virtually avirulent in comparison with phase I organisms. The persistence of viable C. burnetii organisms in the spleen 30 days postinfection is another hallmark of phase I infection, whereas plaque-purified phase II organisms apparently do not survive and viable organisms are not recovered from the spleen (29). Our results confirm these observations. A recently described variant within the Nine Mile strain (9mi/Cr) that possesses an LPS with a structure intermediate between that of phase I and II LPSs (12; H. Mayer, T. Hackstadt, and S. Schramek, unpublished data) was also evaluated here. This mutant was intermediate in virulence between the better characterized phase I and II organisms. Within the limits of the experiment, 9mi/Cr was equal to 9mi/I in its ability to cause fever, but organisms could not be recovered from the spleens of 9mi/Cr-infected animals after 30 days. This failure to detect viable 9mi/Cr organisms in the spleen presents a paradox in that this particular mutant was originally recovered from the placenta of a guinea pig that had been experimentally infected a year previously with 9mi/I (12). It may be that the organisms bearing such a truncated LPS were cleared before they could establish themselves in a persistent state or were sequestered in sites other than the spleen and therefore were not detected.
The comparison of virulence among intrastrain variants of the Nine Mile strain should prove useful in the dissection of the role of the LPS in the pathogenesis of *C. burnetii* infections; however, the presence of these mutants in the environment or their possible role in producing disease has not been established.

The more pressing questions concern the role of phase I LPS variation among strains in the pathogenesis of infection, in particular by those that appear to be associated with endocarditis. For initial evaluation, the Nine Mile strains and the Pris strain were chosen as being typical of acute and endocarditis strains, respectively (11, 33). These strains were selected because they are representative of those groups that appear to be most distinct based on SDS-PAGE profile and antigenicity of their purified LPSs (11) and are currently the best characterized of their respective groups. It should be kept in mind that although there is good reason to suspect that LPS is a major virulence determinant, we cannot rule out other components alone or in association with LPS as determinants of virulence.

While as few as two to four viable organisms of either the 9mi/I or Pris/I strain were sufficient to infect guinea pigs, as determined by seroconversion, virtually all of the animals infected with 9mi/I displayed a fever, while greater than 10⁵ Pris/I organisms were required to induce fever. Stoenner and Lackman (37) have reported the isolation from rodents in the Utah desert of *C. burnetii* that displayed decreased virulence in the guinea pig and that elicited weak antibody responses. The inocula were not extensively quantified in their studies, but it is likely that these strains obtained from rodents were indeed less virulent. Results of our studies provide unequivocal evidence that phase I *C. burnetii* strains can differ considerably in their ability to elicit a febrile response. This observation may relate to the wide spectrum of clinical disease observed in primary Q-fever infection in humans (3, 6, 7, 23, 41), ranging from inapparent to life threatening.

Because the LPS is the predominant surface component that varies among strains, we compared in rabbits the pyrogenic activities of purified 9mi/I and Pris/I LPSs to determine whether the different febrile responses of the two strains could be correlated with pyrogenicity of the LPSs. Consistent with the lack of toxicity in the chick embryo model (12, 34), neither LPS was pyrogenic in this assay.

Our results therefore agree with those from previous studies (2, 34) in which very large quantities of purified *C. burnetii* LPS were required to induce significant fever. It is possible that potent pyrogenic activity resides in a complex of LPS with protein, carbohydrate, or lipid or that the hot phenol purification procedure may alter a pyrogenic component of *Coxiella* LPS. In addition, the responsiveness of the host to the pyrogenic effects of LPS may be altered by *Coxiella* infection (19, 35).

The temporal appearance of phase I and II antibodies elicited by infection with either strain (9mi/I or Pris/I) was typical of the serologic responses of guinea pigs to Q fever. Characteristic of 9mi/I infection (5, 9), Pris/I-infected animals sequentially produced phase II antibodies by 10 to 14 days and phase I antibodies by 21 days.

Various serologic assays failed to demonstrate sufficient specificity for serodiagnosis of the infecting strain. Using sera from experimentally infected guinea pigs, we measured antibodies to surface-exposed antigens on whole cells (MA and MIF assays), to purified LPS (ELISA), and to solubilized whole cells and LPS (immunoblotting). In each of these assays, there was significant cross-reactivity between homologous and heterologous antigens. Experimentally infected guinea pigs did not display specificity in antibody response to the different LPS chemotypes like that observed in hyperimmune rabbit antisera (11). Whether this reflects an inherent difference in the abilities of different species to respond to certain epitopes on *C. burnetii* LPS or simply results from repeated immunization versus natural infection remains to be determined. The serologic responses of guinea pigs appeared to be similar to that of humans with chronic Q fever. These human serum samples displayed a general lack of specificity in response to the different LPSs and considerable individual variation in their response to specific protein components (T. Hackstadt, unpublished data). The development of a specific serodiagnostic test to differentiate among *C. burnetii* strains will likely require a considerable effort to identify unique epitopes on the LPS or other molecules.

Results of our studies indicate that vaccination with either 9mi/I or Pris/I formalin-killed whole cells protects the guinea pig against clinical signs of disease and infection following homologous or heterologous challenge. Viable *C. burnetii* organisms were recovered from the spleens of four of four control, unvaccinated guinea pigs challenged with either 9mi/I or Pris/I. In comparison, viable organisms were detected in the spleen of only one vaccinated guinea pig. This particular animal, which was vaccinated with Pris/I and challenged with 9mi/I, did not display clinical signs (fever) following challenge. Our results are consistent with those of Q-fever vaccine protection studies (4) in domestic livestock in that vaccinated animals were protected against clinical disease, although some proportions still shed the organism in milk and placenta after challenge. Even though viable organisms were not typically found in the spleens of the vaccinated animals in our study, we cannot rule out persistence in other sites. Thus, results of these preliminary experiments indicate a significant degree of cross-protection between vaccine and challenge strains; but definitive statements as to the efficacy of such vaccines as protection against the more serious forms of the disease, i.e., endocarditis, await the development of suitable animal model systems.

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**LITERATURE CITED**


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