Antigenic Variation in the Phase I Lipopolysaccharide of
Coxiella burnetii Isolates

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Coxiella burnetii isolates from a variety of clinical and geographical sources were screened for antigenic variation of lipopolysaccharides (LPSs) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis coupled with silver staining or immunoblotting. All isolates from chronic Q fever or other sources possessed a phase I-type LPS. These LPSs appeared to fall into three groups based on the sodium dodecyl sulfate-polyacrylamide gel electrophoresis profile or on reactivity with rabbit anti-C. burnetii antisera. The LPS of one group was identified on isolates from milk, ticks, or primary Q fever. The two remaining groups were found almost exclusively on isolates from human cases of chronic Q fever.

Coxiella burnetii, the etiologic agent of Q fever, is unique among the rickettsiae in that it undergoes a host-dependent phase variation. Virulent phase I is isolated from nature, whereas the relatively avirulent phase II is selected for during serial laboratory passage in nonimmunologically competent hosts such as eggs or tissue culture (2). Recognized since 1956, phase variation has been defined serologically (25). Compositional analysis of the lipopolysaccharides (LPSs) of phases I and II C. burnetii has demonstrated that this phase variation parallels the smooth-to-rough LPS variation of gram-negative enteric bacteria (22). This intrastrain heterogeneity of LPS structure has been extensively examined. Variation between strains, however, has not been well studied.

We have recently used the procedure of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining of LPS to identify within the Nine Mile strain of C. burnetii a third LPS type believed to be structurally intermediate between the phases I and II LPSs that could not be identified by standard serological methods (8). The variant with the intermediate LPS chemotype was isolated from the placenta of a guinea pig infected almost a year previously with phase I organisms (5). The unusual source of this organism led us to consider (8) potential roles for LPS variation in the establishment of chronic or persistent infections by C. burnetii. To further explore possible roles for LPS variation in the capacity of C. burnetii to establish chronic infections, a number of C. burnetii isolates from chronic Q fever patients and a variety of other sources were screened for antigenic variation of the phase I-type LPS on silver-stained polyacrylamide gels and by immunoblot analysis.

C. burnetii isolates were screened for antigenic or structural variation of the phase I-type LPS on silver-stained polyacrylamide gels (Fig. 1A) and by immunoblotting either with an anti-C. burnetii Nine Mile phase I antiserum (Fig. 1B) or with an anti-C. burnetii Priscilla phase I antiserum (Fig. 1C). The geographical source and passage history of the isolates are listed in Table 1. All isolates possessed a phase I-type LPS that migrated on SDS-PAGE as five or more distinct silver-staining bands in the area of the 14- to 18-kilodalton molecular weight markers, with an indefinite number of faintly staining, but antigenic, bands trailing above (see reference 8 for a description of intrastrain heterogeneity of LPS). It is these slower-migrating and poorly staining LPS species that show the greatest reactivity on immunoblots and differ antigenically between groups. The intensely silver-staining bands which migrate near the 14.3-kDa marker are poorly, or not at all, reactive on immunoblots but are distinctive enough to allow the establishment of three basic groups based on silver-stained polyacrylamide gel profile or on reactivity with rabbit anti-C. burnetii antisera. The LPS of one group was identified on isolates from milk, ticks, or primary Q fever. The two remaining groups were found almost exclusively on isolates from human cases of chronic Q fever.

In some cases the immunoblot detected antigenic differences within groups where the LPSs migrated and stained similarly on SDS-PAGE. For example, the LPS of the Ohio isolate looked very similar to the Nine Mile LPS in silver-stained PAGE profiles, but subtle differences were seen in the antigenicity of some bands by the immunoblot analysis. Similarities in migration of LPS on SDS-PAGE do not necessarily indicate identity, although differences in migration almost certainly indicate structural or chemical heterogeneity.

Previous studies (6, 17) of antigenic variation among C. burnetii isolates could not demonstrate differences between isolates by the serological techniques used. The results described here demonstrate antigenic variation among the LPSs of C. burnetii isolates from nature. All LPSs analyzed from C. burnetii isolates from chronic Q fever patients or from other sources in nature possessed a phase I-type LPS. The isolates examined here, with one exception (Ko), did not appear to consist of a significant proportion of variants possessing either a phase II-type or intermediate-type LPS.
FIG. 1. Silver-stained SDS-PAGE profile (A) and parallel immunoblots (B and C) of the LPSs of C. burnetii isolates. C. burnetii isolates, listed in Table 1, were grown in embryonated chicken eggs and purified by Renografin (E. R. Squibb & Sons, Princeton, N.J.) density gradient centrifugation (31). LPSs were extracted by the hot phenol-water procedure (30) and were subjected to SDS-PAGE on 12.5% polyacrylamide gels as described previously (8, 9), with silver staining by the procedure of Tsai and Frasch (27). Immunoblotting was done as described previously (8, 9), except the antisera used here were produced in rabbits against Formalin-killed intact cells of C. burnetii Nine Mile (B) or C. burnetii Priscilla (C) as follows. Hyperimmune rabbit antisera were prepared against phase I C. burnetii Nine Mile and Priscilla. Briefly, 1 ml of Formalin-killed C. burnetii (1 mg [dry weight] per ml, 0.14 M NaCl) was emulsified in 1 ml of Freund complete adjuvant (Sigma Chemical Co., St. Louis, Mo.) and administered intramuscularly. After 30 days, two intravenous injections of Formalin-killed C. burnetii antigen in 0.14 M NaCl were given. Rabbits were bled 7 days after the last immunizing injection. In panel A, note that the Ko isolate contains a small amount of phase II LPS that migrated near the dye front. Lesser amounts of phase II LPS are visible in the remaining members of group 3. There is an artifact just above the 14.3-kDa marker in panel C, lane Ko, that does not represent an immunoreactive band. Molecular weight markers (kDa) are to the left of panel A.

Based upon silver-stained SDS-PAGE profiles of the purified LPS, it appears that at least three basic groups of phase I-type LPS can be established. One group, typified by the Nine Mile strain of C. burnetii, was comprised of C. burnetii isolates from a variety of sources. The other two groups were primarily from isolations made from chronic Q fever patients. The LPSs of C. burnetii are not chemically characterized well enough to explain the migration patterns or antigenicity of the multitude of bands seen on SDS-PAGE. However, by analogy with SDS-PAGE profiles of the LPS of enteric bacteria (7, 10, 15, 19), the migration of C. burnetii phase I LPS is similar to that of a smooth-type LPS. Like enterobacterial LPS (12), the terminal oligosaccharides of C. burnetii LPS make up the dominant immunogenic and antigenic determinants (23). It is likely, therefore, that the O-antigen equivalents on the LPS of the Priscilla, P, K, and F groups differ from the corresponding structures of the other groups, although some epitopes, particularly those on the faster-migrating LPS species, seem to be shared. Although the technique used here may not detect minor variations, it does establish groups that show major differences in a prominent surface antigen and therefore provides a basis for more detailed structural analysis of phase I LPS variants, comparisons of virulence between strains, and cross-protection experiments between vaccine and challenge strains that vary in this antigenic determinant.

Recently, restriction endonuclease mapping of C. burnetii plasmids has demonstrated an association between plasmid type and disease (21). A clear correlation exists between the various groups established on the basis of plasmid profile (21; L. P. Mallavia, personal communication) and the groups shown here based on LPS profile. The results presented here demonstrate a phenotypic property of C. burnetii isolates obtained from chronic Q fever. It is likely that host factors are involved in the establishment of persistent infection by C. burnetii. However, all isolates from chronic Q fever differed from isolates from primary Q fever or from other sources that are commonly associated with the transmission of Q fever. The implication is that certain strains may be

**TABLE 1. Source and passage history of C. burnetii isolates**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Biological source</th>
<th>Geographical source</th>
<th>Passage history*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nine Mile</td>
<td>Tick</td>
<td>Montana</td>
<td>306GP/ITC/3EP</td>
</tr>
<tr>
<td>Ohio 314</td>
<td>Milk</td>
<td>Ohio</td>
<td>4EP</td>
</tr>
<tr>
<td>California 76</td>
<td>Milk</td>
<td>California</td>
<td>3EP</td>
</tr>
<tr>
<td>El Tayeb</td>
<td>Tick</td>
<td>Egypt</td>
<td>2EP</td>
</tr>
<tr>
<td>Panama</td>
<td>Chigger and mite pool</td>
<td>Panama</td>
<td>4EP</td>
</tr>
<tr>
<td>Priscilla</td>
<td>Placenta, goat</td>
<td>Montana</td>
<td>2EP</td>
</tr>
<tr>
<td>K</td>
<td>Aortic valve, human</td>
<td>Oregon</td>
<td>1GP/2EP</td>
</tr>
<tr>
<td>P</td>
<td>Aortic valve, human</td>
<td>California</td>
<td>2EP</td>
</tr>
<tr>
<td>F</td>
<td>Aortic valve, human</td>
<td>Washington</td>
<td>3EP</td>
</tr>
<tr>
<td>S</td>
<td>Liver, human</td>
<td>Montana</td>
<td>2EP</td>
</tr>
<tr>
<td>Ko</td>
<td>Brachial artery clot, human</td>
<td>Nova Scotia</td>
<td>2EP</td>
</tr>
<tr>
<td>G</td>
<td>Aortic valve, human</td>
<td>Nova Scotia</td>
<td>2EP</td>
</tr>
<tr>
<td>L</td>
<td>Aortic valve, human</td>
<td>Nova Scotia</td>
<td>2EP</td>
</tr>
</tbody>
</table>

* Number of passages in guinea pigs (GP), chicken embryo tissue culture (TC), embryonated chicken eggs (EP), or hamsters (HP).
predisposed toward establishing chronic infections. A single goat isolate, Priscilla, was similar in LPS profile to one of the groups isolated from human Q fever. An isolate possessing an LPS profile similar to that of the other group of chronic Q fever isolates S, Ko, G, and L has not yet been found in sources other than human isolates, but it is probable that similar organisms occur elsewhere in nature. The Nine Mile, Ohio, and California isolates have been studied extensively and are considered as representative of the Q fever agent. The results presented here demonstrate that variants of C. burnetii exist, thus raising the question of the relative proportion of each in different populations.

The finding that the LPS of one group of chronic Q fever isolates was antigenically distinct from the Nine Mile strain LPS presents somewhat of a paradox in that elevated serum immunoglobulins G and A titers against the phase I antigen (LPS) are considered diagnostic of Q fever endocarditis (20, 28), and all patients from which these isolations were made demonstrated high serum antibody titers against phase I C. burnetii Nine Mile (20; M. G. Peacock, unpublished observations). The specificity of the rabbit antiserum used here may be due to the immunization procedure or may reflect different epitopes recognized by humans during chronic infection. Additional work is needed to assess the degree of antigenic cross-reactivity between these LPSs. Despite these considerations, it is clear that one group of C. burnetii isolates possessed an LPS that is antigenically distinct from that of C. burnetii Nine Mile phase I. This observation might explain the occasional reports (18, 26) of Q fever endocarditis without anti-phase I (LPS) antibodies elevated to the titers usually considered as diagnostic.

The infectious nature of the Q fever agent C. burnetii plus increasing concern over the chronic forms of the disease has led to renewed interest in the vaccination of high-risk groups such as laboratory or abattoir workers. A number of vaccine preparations composed of Formalin-killed intact organisms have been tested in humans (1, 13, 14, 24, 29). These vaccines have been believed to be effective and safe as long as skin testing was done and sensitive individuals were excused from vaccination (13). Critical to the success of any vaccine, however, is the degree of antigenic variation in the causative agent(s). LPS appears to be the predominant surface antigen that varies between phases (8). It has been shown that vaccines composed of Formalin-killed phase I cells are 100 to 300 times more vaccinogenic than vaccines composed of phase II cells (16). Recently, this observation has been interpreted as indicating that the phase I antigen (LPS) is the protective immunogen (14). In support of this are demonstrations of protection by trichloroacetic acid (3, 4, 11) and phenol-water-extracted phase I LPS (3). The extent of antigenic variation among isolates of phase I C. burnetii from nature is therefore of additional interest regarding the efficacy of Q fever vaccines.

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LITERATURE CITED


