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Antigenic Variation in the Phase I Lipopolysaccharide of *Coxiella burnetii* Isolates

TED HACKSTADT

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

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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 of these LPSs (Fig. 1A). Minor differences in profile were observed within these groups. Group 1 had LPS that was similar in profile to the LPS of *C. burnetii* Nine Mile. All of these isolates were from primary (acute) Q fever, ticks, or milk and were from various locations around the world. The other two groups were among isolates from chronic Q fever patients. Group 3 was comprised of the S, Ko, G, and L isolates. Group 2, consisting of the Priscilla, P, K, and F isolates, appeared even more distinctive. LPSs from this latter group cross-reacted weakly, or not at all, with an antiserum prepared in rabbits against the Nine Mile strain of *C. burnetii* (Fig. 1B).

The converse was also true. Antiserum against *C. burnetii* Priscilla, chosen as typical of isolates Priscilla, P, K, and F, reacted primarily with LPSs of that class, although a number of putative LPS bands (migrating just above the 18-kDa marker) were recognized among all isolates (Fig. 1C).

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.

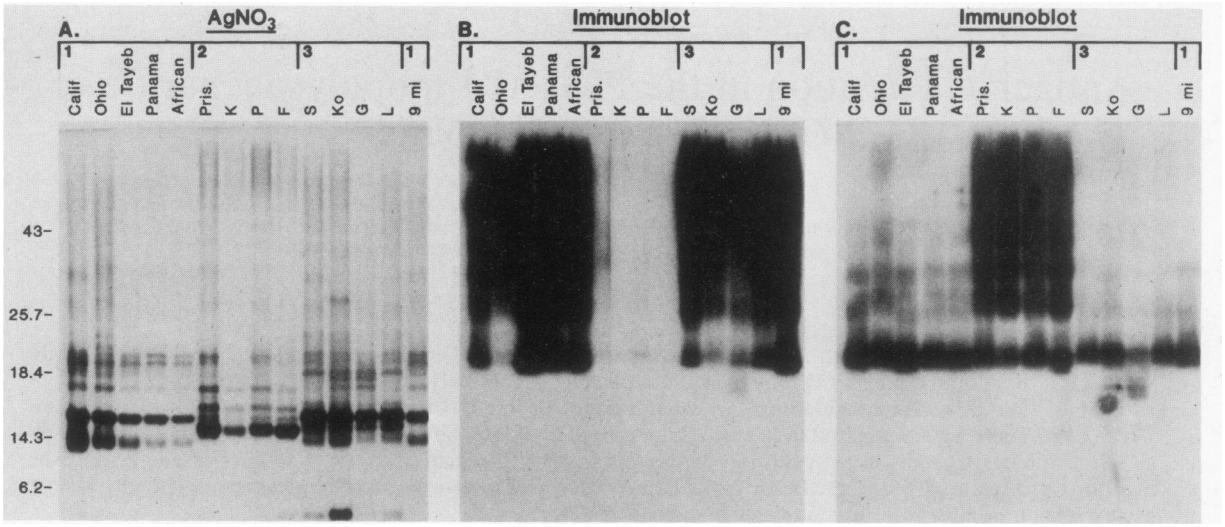

FIG. 1. Silver-stained SDS-PAGE profile (A) and parallel immunoblots (B and C) of the LPSs of *C. burnetii* isolates. *C. burnetii* isolates, listed in Table 1, were grown in embryonated chicken eggs and purified by Renografin (E. R. Squibb & Sons, Princeton, N.J.) density gradient centrifugation (31). 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.

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

Isolate	Biological source	Geographical source	Passage history[a]
Nine Mile	Tick	Montana	306GP/1TC/3EP
Ohio 314	Milk	Ohio	4EP
California 76	Milk	California	3EP
El Tayeb	Tick	Egypt	2EP
Panama	Chigger and mite pool	Panama	4EP
African C9	Blood, human	Central Africa	3HP/4EP
Priscilla	Placenta, goat	Montana	2EP
K	Aortic valve, human	Oregon	1GP/2EP
P	Aortic valve, human	California	2EP
F	Aortic valve, human	Washington	3EP
S	Liver, human	Montana	2EP
Ko	Brachial artery clot, human	Nova Scotia	2EP
G	Aortic valve, human	Nova Scotia	2EP
L	Aortic valve, human	Nova Scotia	2EP

[a] Number of passages in guinea pigs (GP), chicken embryo tissue culture (TC), embryonated chicken eggs (EP), or hamsters (HP).

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

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

1. **Ascher, M. S., M. A. Berman, and R. Ruppanner.** 1983. Initial clinical and immunologic evaluation of a new phase I Q fever vaccine and skin test in humans. J. Infect. Dis. **148**:214–222.
2. **Baca, O. G., and D. Paretsky.** 1983. Q fever and *Coxiella burnetii*: a model for host-parasite interactions. Microbiol. Rev. **47**:127–149.
3. **Brezina, R., and V. Pospisil.** 1970. Study of the antigenic structure of *Coxiella burnetii*. VIII. Immunogenicity of phenol-extracted phase I antigenic component. Acta Virol. **14**:302–306.
4. **Brezina, R., S. Schramek, J. Kazar, and J. Urvolgyi.** 1974. Q fever chemovaccine for human use. Acta Virol. **18**:269.
5. **Fiset, P., R. Ormsbee, R. Silberman, M. Peacock, and S. H. Spielman.** 1969. A microagglutination technique for detection and measurement of rickettsial antibodies. Acta Virol. **13**:60–66.
6. **Fiset, P., D. A. Wike, E. G. Pickens, and R. A. Ormsbee.** 1971. An antigenic comparison of strains of *Coxiella burnetii*. Acta Virol. **15**:161–166.
7. **Goldman, R. C., and L. Leive.** 1980. Heterogeneity of antigenic-side-chain length in lipopolysaccharide from *Escherichia coli* O111 and *Salmonella typhimurium* LT2. Eur. J. Biochem. **107**:145–153.
8. **Hackstadt, T., M. G. Peacock, P. J. Hitchcock, and R. L. Cole.** 1985. Lipopolysaccharide variation in *Coxiella burnetii*: intrastrain heterogeneity in structure and antigenicity. Infect. Immun. **48**:359–365.
9. **Hackstadt, T., W. J. Todd, and H. D. Caldwell.** 1985. Disulfide-mediated interactions of the chlamydial major outer membrane protein: role in the differentiation of chlamydiae? J. Bacteriol. **161**:25–31.
10. **Hitchcock, P. J., and T. M. Brown.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. J. Bacteriol. **154**:269–277.
11. **Kazar, J., R. Brezina, A. Palanova, B. Tvrda, and S. Schramek.** 1982. Immunogenicity and reactogenicity of a Q fever chemovaccine in persons professionally exposed to Q fever in Czechoslovakia. Bull. W.H.O. **60**:389–394.
12. **Lüderitz, O., A. M. Staub, and O. Westphal.** 1966. Immunochemistry of O and R antigens of *Salmonella* and related *Enterobacteriaceae*. Bacteriol. Rev. **30**:192–255.
13. **Luoto, L., J. F. Bell, M. Casey, and D. B. Lackman.** 1963. Q fever vaccination of human volunteers. I. The serologic and skin-test response following subcutaneous injections. Am. J. Hyg. **78**:1–15.
14. **Marmion, B. P., R. A. Ormsbee, M. Kyrkou, J. Wright, D. Worswick, S. Cameron, A. Esterman, B. Ferry, and W. Collins.** 1984. Vaccine prophylaxis of abattoir-associated Q fever. Lancet **ii**:1411–1414.
15. **Munford, R. S., C. L. Hall, and P. D. Rick.** 1980. Size heterogeneity of *Salmonella typhimurium* lipopolysaccharides in outer membranes and culture supernatant membrane fragments. J. Bacteriol. **144**:630–640.
16. **Ormsbee, R. A., E. J. Bell, D. B. Lackman, and G. Tallent.** 1964. The influence of phase on the protective potency of Q fever vaccine. J. Immunol. **92**:404–412.
17. **Ormsbee, R. A., E. G. Pickens, and D. B. Lackman.** 1964. An antigenic analysis of three strains of *Coxiella burnetii*. Am. J. Hyg. **79**:154–162.
18. **Palmer, S. R., and S. E. J. Young.** 1982. Q fever encodarditis in England and Wales, 1975–81. Lancet **ii**:1448–1449.
19. **Palva, E. T., and P. H. Makela.** 1980. Lipopolysaccharide heterogeneity in *Salmonella typhimurium* analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Eur. J. Biochem. **107**:137–143.
20. **Peacock, M. G., R. N. Philip, J. C. Williams, and R. S. Faulkner.** 1983. Serological evaluation of Q fever in humans: enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. Infect. Immun. **41**:1089–1098.
21. **Samuel, J. E., M. E. Frazier, and L. P. Mallavia.** 1985. Correlation of plasmid type and disease caused by *Coxiella burnetii*. Infect. Immun. **49**:775–779.
22. **Schramek, S., and H. Mayer.** 1982. Different sugar compositions of lipopolysaccharides isolated from phase I and pure phase II cells of *Coxiella burnetii*. Infect. Immun. **38**:53–57.
23. **Schramek, S., J. Radziejewska-Lebrecht, and H. Mayer.** 1985. 3-C-branched aldoses in lipopolysaccharide of phase I *Coxiella burnetii* and their role as immunodominant factors. Eur. J. Biochem. **148**:455–461.

24. **Smadel, J. E., M. J. Snyder, and F. C. Robbins.** 1948. Vaccination against Q fever. Am. J. Hyg. **47**:71–81.
25. **Stoker, M. G. P., and P. Fiset.** 1956. Phase variation of the Nine Mile and other strains of *Rickettsia burnetii*. Can. J. Microbiol. **2**:310–321.
26. **Tobin, M. J., N. Cahill, G. Gearty, B. Maurer, S. Blake, K. Daly, and R. Hone.** 1982. Q fever endocarditis. Am. J. Med. **72**:396–400.
27. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
28. **Turck, W. P. G., G. Howitt, L. A. Turnberg, H. Fox, M. Longson, M. B. Matthews, and R. Das Gupta.** 1976. Chronic Q fever. Q. J. Med. **45**:193–217.
29. **Vivona, S., J. P. Lowenthal, S. Berman, A. S. Benenson, and J. E. Smadel.** 1964. Report of a field study with Q fever vaccine. Am. J. Hyg. **79**:143–153.
30. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. Methods Carbohydr. Chem. **5**:83–93.
31. **Williams, J. C., M. G. Peacock, and T. F. McCaul.** 1981. Immunological and biological characterization of *Coxiella burnetii*, phases I and II, separated from host components. Infect. Immun. **32**:840–851.