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Contract Title: Studies of the Outer Membrane Proteins of Campylobacter Jejuni for Vaccine Development

Approved for public release; distribution unlimited

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JUN 07 1993
93-12620

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Unclassified

Unclassified

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Unclassified

93-12620
Lipopolysaccharide Structures of Campylobacter fetus Are Related to Heat-Stable Serogroups

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Received 1 August 1985/Accepted 5 October 1985

To determine whether lipopolysaccharide (LPS) structures of Campylobacter fetus are related to the three known heat-stable serogroups, proteinase K-treated whole cell lysates obtained from strains of each serogroup were electrophoresed in polyacrylamide gels. All strains had smooth-type LPS with multiple high-molecular-weight repeating units. The profiles of serogroup A from C. fetus subsp. fetus and from C. fetus subsp. venerealis were identical, but they were different from those of C. fetus subsp. fetus serogroups B and AB. When we immunoblotted the LPS of these serogroups with normal or immune rabbit serum we found homologous recognition between serogroups A from C. fetus subsp. fetus and C. fetus subsp. venerealis. Similarly, serogroups AB and B from C. fetus subsp. fetus showed homologous recognition. However, antiserum against serogroup A did not recognize serogroups B and AB and vice versa. Absorption studies confirmed the identity of LPS from all serogroup A C. fetus strains and cross-reactivity of the serogroup B and AB strains with one another. Serogroup A strains were resistant to the bactericidal activity in normal human serum, whereas serogroup B and AB strains generally were susceptible; isolates from humans predominantly belonged to serogroup A. Results of these studies suggest that the LPS composition forms the basis for the heat-stable serotyping system for C. fetus and that the structural and antigenic variants are associated with differential serum susceptibility.

Campylobacter fetus (formerly known as Vibrio fetus) strains have been known as veterinary pathogens since the turn of the century and as human pathogens since 1947 (11). Two subspecies now are recognized: C. fetus subsp. venerealis, which causes infectious abortion and sterility in cattle and probably does not cause disease in humans (7), and C. fetus subsp. fetus, which causes infectious abortion in sheep and cattle (10) and systemic infections in humans (5).

When we recently (8) examined the lipopolysaccharide (LPS) characteristics of C. fetus by polyacrylamide gel electrophoresis, the LPS structures that were resolved showed minimal core regions and several high-molecular-weight complexes. In light of results of the previous work, and in analogy with members of the family Enterobacteriaceae, we thought that the LPS molecules may provide the basis for the previous typing of C. fetus into the three different serogroups described by Berg et al. (1). In addition, that they had distinct but closely related LPSs (9) also could explain the serologic similarities. In this study, using immunoblotting techniques, we investigated C. fetus strains to see whether LPS antigens are responsible for the observed serologic differences and cross-reactions.

MATERIALS AND METHODS

The C. fetus strains used in this study were from the culture collections of the Denver Veterans Administration Medical Center Campylobacter Laboratory and the Agricultural Research Service National Animal Disease Laboratory (Table 1); they were identified and passaged and stored as described previously (8). Cells of serogroup A, B, or AB from standard reference Campylobacter strains were harvested, washed, heated in steam, and suspended as described previously (8). Polyacrylamide gel electrophoresis was performed by the use of a modified Laemmli buffer system as described previously (2). After electrophoresis, gels were fixed, and LPS was resolved as described by Hitchcock and Brown (4). A Western blot procedure described previously (9) was used.

The susceptibility of the test Campylobacter strains to the bactericidal activity present in normal human serum was assessed in a standardized assay as described previously (3).

RESULTS

Polyacrylamide gel electrophoresis of proteinase K-treated whole cell lysates. We compared the polyacrylamide gel electrophoresis profiles of the proteinase K-treated whole cell lysates (4) of four heat-stable serogroups. Proteinase K-treated whole cell lysates from C. jejuni serogroup C showed a rough-type LPS profile with no high-molecular-weight complexes (data not shown), as has been described previously (8). The profiles of C. fetus LPS obtained from each set of three strains with the same serotype were similar to one another (Fig. 1). All had smooth-type LPS with
multiple high-molecular-weight repeating units. The profiles of serogroup A from C. fetus subsp. venericaudis and from C. fetus subsp. fetus were identical (Fig. 1, lanes a through f). The profiles of C. fetus serogroups B and AB were different from those of C. fetus serogroup A, with the high-molecular-weight complexes in serogroup B and AB strain (Fig. 1, lane g through l) migrating slightly more rapidly than those in the serogroup A strains.

**Western blots of C. fetus LPS with Campylobacter immune serum.** By immunoblotting the proteinase K-treated whole cell lysates, we were able to study antigenic relationships of LPS from the three heat-stable serogroups A, B, and AB. Using this method, when we immunoblotted the LPS of these serogroups with normal or immune rabbit raised against the three serogroups, we found homologous recognition between serogroups A from C. fetus subsp. fetus and C. fetus subsp. venericaudis (Fig. 2). Similarly, serogroup AB and B from C. fetus subsp. fetus showed homologous recognition. However, antisera against serogroup A did not recognize serogroups B and AB and vice versa. Minimal or no recognition was observed by normal rabbit serum. Absorption studies confirmed the identity of LPS from all serogroup A C. fetus strains and cross-reactivity of the serogroup B and AB strains with one another (data not shown). By immunoblotting the proteinase K-treated whole cell lysates of several C. fetus strains of human origin that had not been previously serogrouped, we found that three of these strains (80-109, 82-40, and 84-97) were recognized by antisera to serogroup A, and one strain (83-88) was recognized by antisera to serogroup B and less strongly by antisera to serogroup AB (data not shown). These observations were confirmed later when those strains were serotyped by the classical slide agglutination technique (1).

**Serum susceptibility to normal human serum.** In a preliminary analysis, a serogroup A isolate (84-112) was resistant to serum (0.06 log10 killing), whereas one serogroup AB (84-91) and two serogroup B (83-88 and 84-90) isolates were susceptible to serum with greater than 2 log10 (99%) killing. Of 15 human isolates studied, 9 were serogroup A, 4 were
serogroup B, and 2 were serogroup AB (Table 1). Six serogroup A strains were resistant to serum and two were
intermediately susceptible to serum. Both of the serogroup AB strains were susceptible to serum, and four of
the serogroup B strains were susceptible to serum and one was
intermediately susceptible to serum.

DISCUSSION

Although most C. fetus isolates from humans and animals
have a smooth-type LPS with multiple high-molecular-
weight O-antigen polysaccharide side chains, for the purpose
of exploring the molecular basis of the heat-stable antigens
of C. fetus (6), we selected three strains of each serogroup
(C. fetus subsp. fetus serogroups A, B, and AB; C. fetus
subsp. venerealis serogroup A; and C. jejuni serogroup C).
No marked variation was found in the LPS profiles within
each serogroup. Profiles of C. jejuni serogroup C were
markedly different from those of C. fetus, confirming our
earlier observations with strains that had not been
serogrouped (8, 9), and the LPS profiles of strains of
serogroups B and AB were slightly different than those
of serogroup A strains. In contrast, identical LPS profiles were
found in C. fetus subsp. fetus serogroup A and C. fetus
subsp. venerealis serogroup A, which were consistent with
the earlier agglutination test results (1).

By immunoblotting C. fetus strains, we found that the LPS
structures were recognized by the homologous antiserum used
in serogrouping, suggesting that LPS antigens may be the
basis for the serogrouping system of Berg et al. (11). Antisera
from two C. fetus serogroup A strains were able to recognize
the high-molecular-weight polysaccharide side chains of all
the strains which belong to serogroup A. However, these
antisera did not recognize LPS antigens of C. fetus strains of
serogroups B and AB and vice versa. We also found that
immune serum raised to C. fetus serogroup B whole cells
recognized the core structures of homologous and heterolo-
gous C. fetus strains to different degrees. These observations
confirm results of previous studies (9) that the LPS core
structures of C. fetus strains share antigenic determinants
with one another and that these core structures in serogroup
A strains are hidden from the cell surface: i.e., they are
unrecognized by immune homologous serum raised against
whole cells.

Results of previous studies have shown that there is a
relationship between LPS structure and serum susceptibility
of campylobacters (9). We have found in this study that none
of the C. fetus strains of serogroup A, including both animal
and human isolates, were susceptible to serum. In contrast,
the C. fetus strains of serogroups B or AB were all suscepti-
bile or intermediately susceptible to serum. Because 23 of
27 C. fetus isolates from humans in a previous study were
resistant to serum and 3 were intermediately susceptible to
serum (M. J. Blaser, P. F. Smith, and J. A. Hopkins, unpublished data). Although in the

This work was supported in part by the Pan American Health
Organization, the U.S. Army Medical Research and Development
Command, and the Medical Research Service of the Veterans
Administration.

ACKNOWLEDGMENTS

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