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

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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 (1) for use for injection into rabbits and as slide agglutination antigens. Rabbit antisera were produced as described previously (1).

Antisera were absorbed with homologous or heterologous live cells by the addition of 10^{10} packed cells to undiluted serum and by incubation of the suspension for 1 h at 37°C. The cells then were removed by centrifugation at 12,000 × *g* for 15 min, and the supernatant was reabsorbed 5 times.

We used the method of Hitchcock and Brown (4) with minor modifications to detect LPS in whole cells 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

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TABLE 1. *C. fetus* strains used in LPS studies

VA strain no.[a]	Subspecies	Source	Site	Serogroup	Log_{10} kill[b]
84-92 (1254)	*fetus*	Bovine	Fetus	A	0.05
84-93 (447)	*fetus*	Human	Cerebrospinal fluid	A	0.38
84-97 (1125)	*fetus*	Human	Blood	A	ND[c]
84-110 (469)	*venerealis*	Bovine	Fetus	A	ND
84-111 (998)	*venerealis*	Bovine	Genital secretion	A	ND
84-112 (1002)	*venerealis*	Bovine	Genital secretion	A	0.06
84-86 (1284)	*fetus*	Human	Blood	A	0.05
84-88 (1286)	*fetus*	Human	Blood	A	0.16
82-40	*fetus*	Human	Blood	A	0.05
80-109	*fetus*	Human	Blood	A	0.05
84-103 (1271)	*fetus*	Human	Blood	A	0.2
81-173	*fetus*	Human	Cerebrospinal fluid	A	0.05
81-200	*fetus*	Human	Feces	A	0.05
84-104 (1126)	*fetus*	Monkey	Blood	AB	ND
84-91 (1366)	*fetus*	Human	Blood	AB	2.96
84-94 (1367)	*fetus*	Human	Blood	AB	2.70
84-10 (1137)	*fetus*	Human	Blood	B	2.56
84-90 (1083)	*fetus*	Bovine	Fetus	B	3.93
84-87 (1275)	*fetus*	Human	Blood	B	0.4
84-107 (1127)	*fetus*	Human	Blood	B	2.71
83-88	*fetus*	Human	Blood	B	1.63

[a] VA, Veterans Administration. Numbers in parentheses are the National Animal Disease Center strain number.
[b] In standardized bactericidal assay (3).
[c] ND, Not determined.

multiple high-molecular-weight repeating units. The profiles of serogroup A from *C. fetus* subsp. *venerealis* 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 strains (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. *venerealis* (Fig. 2). Similarly, serogroup 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. 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 antiserum to serogroup A, and one strain (83-88) was recognized by antiserum to serogroup B and less strongly by antiserum 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 log_{10} 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 log_{10} (99%) killing. Of 15 human isolates studied, 9 were serogroup A, 4 were

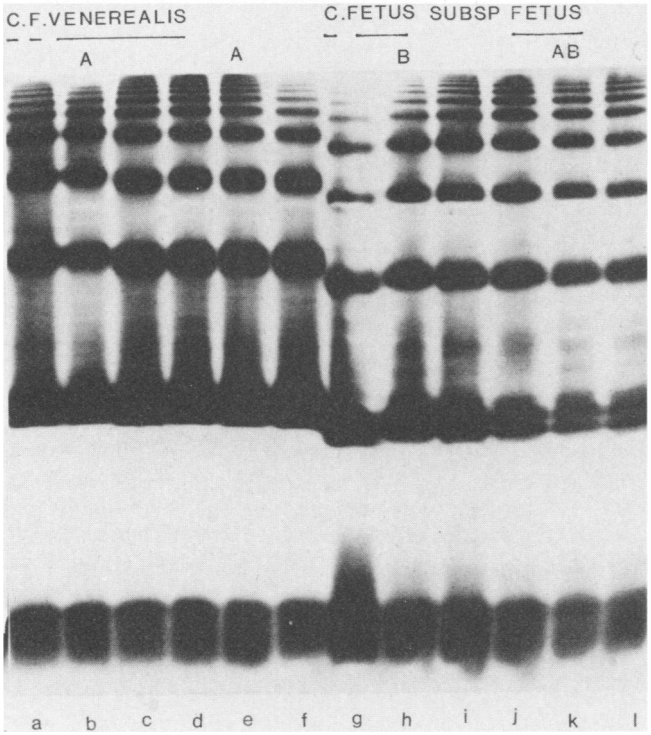

FIG. 1. Silver stain of 15% polyacrylamide gel with proteinase K-treated whole cell lysates from *C. fetus*. Lanes a through c, *C. fetus* (C.F.) subsp. *venerealis* serotype A strains 84-110, 84-111, and 84-112, respectively; lanes d through f, *C. fetus* subsp. *fetus* serotype A strains 84-92, 84-93, and 84-97, respectively; lanes g through i, *C. fetus* subsp. *fetus* serotype B strains 84-90, 84-107, and 84-108, respectively; lanes j through l, *C. fetus* subsp. *fetus* serotype AB strains 84-91, 84-94, and 84-104, respectively.

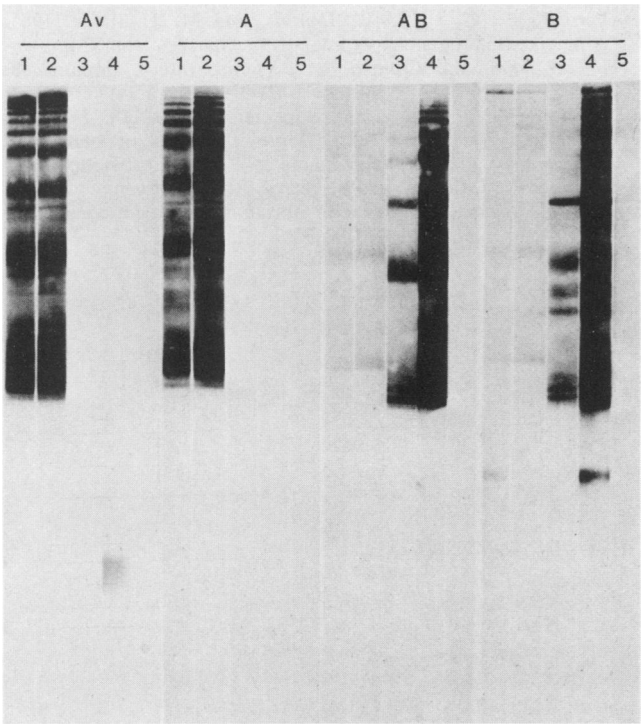

FIG. 2. Western blot of proteinase K-treated whole cell lysate preparations of *C. fetus* subsp. *venerealis* serotype A strain 84-110 (Av), *C. fetus* subsp. *fetus* serotype A strain 84-93 (A), *C. fetus* subsp. *fetus* serotype AB strain 84-91 (AB), and *C. fetus* subsp. *fetus* serotype B strain 84-90 (B) with the following immune rabbit sera: *C. fetus* subsp. *venerealis* serotype A strain 84-110 (lanes 1), *C. fetus* subsp. *fetus* serotype A strain 84-93 (lanes 2), *C. fetus* subsp. *fetus* serotype AB strain 84-107 (lanes 3), *C. fetus* subsp. *fetus* serotype B strain 84-104 (lanes 4), and unimmunized (lanes 5). All sera were diluted 1:100, except antiserum to strain 84-107, which was diluted 1:50.

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 antisera used in serogrouping, suggesting that LPS antigens may be the basis for the serogrouping system of Berg et al. (1). 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, those 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 heterologous *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 susceptible 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, J. A. Hopkins, I. Heinzer, and W.-L. L. Wang, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 262, 1984), it seems likely that most isolates from humans belong to serogroup A. As part of the design of the present study, we sought serogroup B and AB *C. fetus* isolates from humans to test their susceptibility to serum and thus probably overrepresented their proportion among isolates from humans.

In preliminary studies, using ^{32}P-labeled LPS, we found that a serum-susceptible serogroup B strain produced LPS molecules with a smaller proportion of high-molecular-weight polysaccharide side chains than did two serum-resistant serogroup A strains (M. J. Blaser, G. Perez-Perez, and J. A. Hopkins, unpublished data). Although in the present study there was some overlap between serogroup A and serogroups B and AB strains, results suggest that the structural and antigenic variants of LPS are associated with differential susceptibility to serum, and these differences possibly may be related to virulence (3, 9).

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