Conservation and Diversity of Campylobacter pyloridis Major Antigens

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Infection with Campylobacter pyloridis has been strongly associated with gastritis in humans although its etiologic significance is currently undefined. We examined the structure and antigenicity of whole-cell, outer-membrane, acid-extractable surface protein, and proteinase K-treated whole-cell lysate preparations from eight C. pyloridis strains by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting with homologous and heterologous immune rabbit serum. Whole-cell and outer-membrane profiles observed in all strains of C. pyloridis were nearly identical; none were similar to those of C. jejuni and C. fetus. Major whole-cell bands migrated at 26,000, 29,000, 56,000, and 62,000 molecular weights. The acid-extracted protein profiles of all C. pyloridis strains were also not to one another and showed similarities with acid-extracted proteins from C. jejuni, with major bands migrating at 29,000, 48,000 to 53,000, and 62,000. All proteinase K-treated lysates showed different lipopolysaccharide (LPS) profiles, ranging from rough to smooth with multiple repeating side chains. Immunoblots of whole-cell and proteinase K-treated preparations of the C. pyloridis showed that there was antigenic cross-reactivity of proteins migrating at 62,000 and 56,000, but not in other regions, and cross-reactivity between LPS core regions but not side chains. These results suggest that C. pyloridis has both protein and core LPS group antigens and strain-specific protein and LPS side chain antigens.

Campylobacter pyloridis has been cultured from gastric biopsy samples from a high percentage of patients with active gastritis, benign gastric ulcers, or duodenal ulcers (8, 16, 25). Whether C. pyloridis causes or contributes to these disorders or whether its presence reflects secondary colonization is unknown at present. At the least, the presence of C. pyloridis or closely related organisms (30) represents an important marker for persons at risk for these inflammatory conditions.

However, the organism grows slowly on laboratory media, and the diagnosis of gastric C. pyloridis infection may be delayed if culturing is used (L. L. Walters, R. E. Budin, and G. Paull, Letter, Lancet i:42, 1986). In most cases, these organisms also can be seen on histological examination of inflamed gastric epithelium with appropriate stains (8), but the possibility of replacing endoscopic biopsy with a serologic assay for diagnosis of active gastritis would be a major advance (J. Eldridge, A. M. Lessells, and D. J. Jones, Letter, Lancet i:1237, 1984). Several groups of investigators are developing such serological assays; however, the antigens have not been defined (B. J. Marshall, D. B. McGechie, G. J. Francis, and P. J. Utley, Letter, Lancet i:281, 1984; B. J. Rathbone, J. I. Wyatt, B. W. Worsley, L. K. Trejosiwicz, R. V. Heatley, and M. S. Losowsky, Letter, Lancet i:1217, 1985). Similarly, little work has been done to characterize the major antigens for Campylobacter pyloridis and to define the relationships among various isolates (18, 25). Such information might be used to differentiate among C. pyloridis isolates to form the basis for a serotyping system, or it may correlate with different pathogenic properties of the strains as has been observed for Escherichia coli, for example (10). Conversely, recognition of a common protein may provide a group antigen that can be used for serological assays or other diagnostic purposes.

For these reasons, we studied C. pyloridis strains by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to identify the protein and lipopolysaccharide (LPS) profiles. To further define the antigenic relationships among several C. pyloridis strains, we used enzyme-linked immunosorbent assay (ELISA) and immunoblotting techniques. We found that C. pyloridis outer-membrane and whole-cell profiles were similar but not identical and that there was great variation in LPS profiles, observations that were confirmed and extended by immunoblotting.

MATERIALS AND METHODS

Bacterial strains. The C. pyloridis strains used in this study were obtained from the culture collection of the Denver Veterans Administration Medical Center Campylobacter laboratory (Table 1). All strains used in this study had been identified as C. pyloridis by the following criteria: isolation site, atmosphere required for growth, incubation time, morphology on Gram stain, and rapid urease test (18). Most of the strains had been passaged at least five times on sheep blood agar plates (PASCO, Wheat Ridge, Colo.). All of the strains were maintained frozen at −70°C in brucella broth containing 15% glycerol. Working stocks of strains were obtained by culturing the freezer stock on chocolate agar at 37°C in a microaerobic atmosphere containing 7.5% hydrogen, 7.5% carbon dioxide, 5% oxygen, and 80% nitrogen. Cells were harvested after 5 to 7 days of incubation in sterile distilled water and centrifuged twice at 3,000 × g for 20 min, and the pellet was frozen at −20°C until the cells were used. For comparison, we used four previously studied (27) strains of other Campylobacter species (C. jejuni 79-193 and 83-85 and C. fetus 81-200 and 82-40) pathogenic for humans; these strains were treated as described above except that they were incubated for 48 h. A smooth wild-type strain of Salmonella minnesota (WT 218) also was used as a control.
TABLE 1. Identity and phenotypic properties of the C. pyloridis strains used in this studya

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin</th>
<th>Susceptibility to:</th>
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<tbody>
<tr>
<td>VA 84-180</td>
<td>Texas</td>
<td>S</td>
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<tr>
<td>VA 84-181</td>
<td>Texas</td>
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<tr>
<td>VA 84-182</td>
<td>Texas</td>
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<tr>
<td>VA 84-183</td>
<td>Texas</td>
<td>S</td>
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<tr>
<td>VA 85-456</td>
<td>Australia</td>
<td>S</td>
<td>R</td>
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<tr>
<td>VA 86-63</td>
<td>New York</td>
<td>S</td>
<td>R</td>
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<tr>
<td>VA 86-86</td>
<td>New York</td>
<td>S</td>
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<tr>
<td>VA 86-130</td>
<td>New York</td>
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<td>R</td>
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a All of the isolates used were from human gastric biopsies, all were negative by hippurate hydrolysis and nitrate reductase tests and positive by the rapid urease test, and all grew optimally at 37°C.

Antigen preparations. Preparations of whole cells were solubilized as previously described (3), and sodium lauryl sarcosinate (Sarkosyl; CIBA-GEIGY Corp. Summit, N.J.) treatment for the preparation of outer-membrane proteins of Campylobacter species also has been described previously (3). We treated whole-cell lysates with proteinase K by the method of Hitchcock and Brown (9) with minor modifications to resolve lipopolysaccharide as previously described (27). Whole cells were suspended in sample buffer and then boiled at 100° C for 5 min before being applied to the gel. We extracted surface proteins from C. pyloridis strains with glycine buffer (pH 2.2) by the methods of McCoy et al. (17) as previously described (2). Protein concentrations were measured by the Markwell et al. modification of the Lowry method for membrane proteins (14).

SDS-PAGE. Protein profiles of each of the glycine (acid)-extracted, whole-cell, and membrane preparations were examined by discontinuous SDS-PAGE as previously described (3). Samples with 1 to 2 μg of protein were applied to each gel lane. After electrophoresis, gels were fixed and proteins were resolved by the modified silver stain of Oakley et al. (22). For proteinase K-treated whole-cell lysates, PAGE without SDS was performed by a modification of the system of Laemmli as described previously (27). After electrophoresis, gels were fixed and LPS was resolved with a silver stain as reported by Hitchcock and Brown (9).

Production of antiserum. Adult New Zealand White rabbits (two) were immunized with 10⁸ C. pyloridis cells per ml in the form of Formalin-killed suspensions to produce OH antiserum or boiled suspensions to produce O antiserum as previously described for the Enterobacteriaceae (7). The rabbits were inoculated intravenously on days 0, 5, 8, 14, 22, and 32 with increasing volumes of the antigens from 0.25 to 2.0 ml. On day 40, the rabbits were bled and the sera were collected and stored at −70°C. Titers of preimmune and immune sera were determined by an ELISA as previously described (2). Briefly, we used as the antigen a pool of sonicates from five C. pyloridis strains (84-180, 84-182, 84-183, 86-63, and 86-86) at a final concentration of 0.9 μg per well, and the detection antibody was horseradish peroxidase-conjugated swine anti-rabbit immunoglobulins (Accurate Chemicals, Westbury, N.Y.) diluted 1:1,500. Based on results in this assay with normal rabbit serum, we defined a positive titer as a serum dilution showing an optical density reading greater than 0.100.

Immunoblot procedures. The Western blot procedure we used was as previously described (28). Gels of whole or proteinase K-treated cells from C. pyloridis strains were transferred to nitrocellulose paper by electroblotting for 12 h at 100 mA. After nonspecific binding was blocked with milk-borate buffer, the nitrocellulose paper was incubated at 25°C for 4 h in a 1:100 dilution of the test serum sample. After being washed, the nitrocellulose paper was incubated at 25°C for 2 h with a 1:1,500 dilution of horseradish peroxidase-conjugated swine anti-rabbit immunoglobulins (Accurate Chemicals). The results that we observed were obtained with both rabbits in each pair, but to avoid repetition we only show data on one. We performed immunoblots on all eight strains. However, most of the information is presented on only four or five strains because we found similar results for the other strains.

RESULTS

Characterization of whole-cell preparations in SDS-PAGE. The profiles of C. jejuni, C. fetus, and the six C. pyloridis strains in SDS-PAGE are shown in Fig. 1. All six C. pyloridis strains showed similar whole-cell profiles that were substantially different from those of the C. jejuni and C. fetus preparations. There were seven major bands (molecular weights, 62,000, 56,000, 53,000, 48,000, 44,000, 29,000, and 26,000) always present, and there was considerable variation among the minor bands. Only one major band, migrating at
62,000, was shared by the three Campylobacter species which for C. jejuni and C. fetus correspond to the major flagellar protein (4).

**SDS-PAGE of acid-extracted preparations.** The SDS-PAGE profiles of the acid-extracted material from the five strains of C. pyloridis used were similar to one another (Fig. 2). There were four major bands observed in all five strains which also corresponded to four of the seven major bands observed in the whole-cell preparations (molecular weights, 62,000, 53,000, 48,000, and about 30,000). Two other major bands (56,000 and 44,000) were not present in one strain (C. pyloridis 84-180). The profiles shown were similar to the profiles of acid-extracted material from C. jejuni, with major bands migrating at 63,000, 44,000, and 29,000 (2). Differences were found in the number and migration patterns of the minor bands among the C. pyloridis strains.

**Characterization of detergent-insoluble membrane preparations.** We next examined the protein profiles seen in the Sarkosyl-insoluble outer-membrane preparations of the same C. pyloridis strains (Fig. 3). All five strains showed a doublet of major bands migrating between 52,000 and 57,000. Two of five strains also showed a major band migrating at 44,000. When the entire profiles from the five isolates were considered, strains 84-181 and 84-182 (lanes b and c) appeared identical, strains 84-180 and 85-456 (lanes a and e) appeared similar to one another but different from the first two strains, and strain 84-183 (lane d) was substantially different from the other four strains. Although several of the major bands were similar, C. pyloridis could be readily distinguished from C. fetus, C. faecalis, and thermophilic species (C. jejuni and C. coli) on the basis of outer-membrane profiles (data not shown).

**PAGE of proteinase K-treated whole-cell lysates.** Using whole-cell lysates treated with proteinase K to resolve LPS, we next compared the PAGE profiles from the five strains of C. pyloridis with the profiles from C. jejuni, C. fetus, and S. minnesota (Fig. 4). The LPS profiles obtained from the five strains of C. pyloridis showed great variability; they ranged from a ladderlike profile (lane e) characteristic of smooth strains of the Enterobacteriaceae to a classical rough-type profile (lane c) similar to rough (Ra) strains of S. minnesota (9); these fast-migrating LPS molecules resemble those observed in C. jejuni (27). This wide variety of LPS profiles shown for C. pyloridis contrasts with the relatively uniform profiles shown for C. jejuni and C. fetus LPS illustrated in lanes g and h (27) and also contrasts with the uniform profile observed in whole-cell and acid-extractable preparations from the same C. pyloridis strains. The LPS profile obtained in all C. pyloridis strains contained one to three argentophilic bands which migrated somewhat faster than a protein standard of molecular weight 14,000 (data not shown) and probably correspond to the core region as further suggested.

![Fig. 2](http://iai.asm.org/)

**FIG. 2.** SDS-PAGE (on 10% acrylamide) of acid-extracted preparations of C. pyloridis. The methods used were as described in the legend to Fig. 1. The following strains were used (lanes): a, 84-180; b, 84-181; c, 84-182; d, 84-183; and e, 85-456. The numbers on the left indicate molecular weight in thousands.

![Fig. 3](http://iai.asm.org/)

**FIG. 3.** SDS-PAGE (on 10% acrylamide) of Sarkosyl-insoluble (outer) membranes of five C. pyloridis strains. The preparations examined were from the following strains (lanes): a, 84-180; b, 84-181; c, 84-182; d, 84-183; and e, 84-456. The numbers on the left indicate molecular weight in thousands.
86-86; preparations from these three strains showed the same recognition by the four different OH antisera shown in lanes b to e. All showed the strongest recognition by 84-182 (lane c) and 86-63 (lane e) OH antisera and weaker recognition by 84-183 (lane d) OH antisera. C. pyloridis 84-180 is representative of a second pattern, which also includes C. pyloridis 84-183; by immunoblotting, both showed similar profiles in the region below 31,000. As with the previous pattern, the strongest recognition was shown by 84-182 (lane c) and 86-63 OH (lane e) antisera. However, the weakest recognition was by 84-181 OH (lane b) antisera. Finally, C. pyloridis 85-456 and 86-63 showed patterns different from one another and from the previous patterns. However, a common observation was the stronger recognition showed by 86-63 OH (lane e) than by 84-183 OH (lane d) antisera. In all of the immunoblots, the two most prominent regions of recognition were of molecular weights between 66,000 and 45,000 and the region below 31,000. Using sera from rabbits immunized with C. jejuni or C. fetus cells, we observed cross-reacting antibodies that recognized the 62,000 band of C. pyloridis 84-182 (arrow); this band corresponds with the major flagellar antigen of C. jejuni and C. fetus (4). To determine whether the 62,000 band of C. pyloridis also

FIG. 4. PAGE (on 15% acrylamide) of proteinase K-treated whole-cell lysates of (lanes) a, S. minnesota WT 218; b, C. pyloridis 84-180; c, C. pyloridis 84-181; d, C. pyloridis 84-182; e, C. pyloridis 84-183; f, C. pyloridis 85-456; g, C. jejuni 79-193; and h, C. fetus 82-40. The gels were silver stained as previously described (27).

by subsequent immunoblot experiments. We evaluated the possibility that the great variability in the LPS profiles was related to the numerous in vitro passages of the strains studied. Examination of four fresh clinical isolates from Denver that had three or fewer in vitro passages showed a similar diversity of LPS profiles.

ELISA of immune C. pyloridis serum to a pooled C. pyloridis antigen. To evaluate the antisera obtained after immunization of rabbits with boiled (O) for Formalin-treated (OH) C. pyloridis cells, we used an ELISA with sonicates of five C. pyloridis strains as the antigen. We tested the O antisera of five different C. pyloridis strains, of which three were in the pool and two were not. All showed titers greater than 1:3,200 (Fig. 5A). The OH antisera, three against homologous strains and one against a heterologous strain, also showed titers greater than 1:3,200 (Fig. 5B).

Immunoblots of C. pyloridis whole cells with Campylobacter immune serum. By immunoblotting whole cells, we were able to study antigenicity of proteins from a variety of strains of C. pyloridis. With this method, there was minimal or no recognition of antigens with normal rabbit serum (Fig. 6, lane a). In contrast, OH immune serum raised to C. fetus and C. jejuni recognized a band at about 62,000 (arrow) (Fig. 6, lanes g and h). The homologous O antisera recognized a large number of bands but always fewer than when we used the homologous OH antisera, suggesting that some protein antigens on C. pyloridis may be heat-stable or internal. Figure 6 shows four different patterns observed when C. pyloridis whole-cell preparations were used as the antigen. C. pyloridis 84-182 is representative of the first pattern, which also includes C. pyloridis 86-130 and C. pyloridis
A flagellar protein, we used serum from a rabbit
immunized with purified flagellae from C. jejuni (4); this
serum recognized the 62,000 band, as well as a poorly
resolved region between 31,000 to 25,000 (data not shown).

**Immunoblots of C. pyloridis LPS with Campylobacter immune serum.** Figure 7 shows the homologous recognition in
immunoblots of five different (representative) C. pyloridis
LPS preparations with the homologous rabbit serum. There
is a good correlation between the profiles observed in the
silver stain (Fig. 4) and the immunoblots for strains 84-180
(lane a) and 84-183 (lane d). In contrast, for C. pyloridis
strains 84-181, 84-182, and 85-456 the only recognition was in
the low-molecular-weight region. We found no recognition of any of these preparations by normal rabbit serum and
minimal recognition with C. fetus and C. jejuni O immune
serum (data not shown). By immunoblotting with antisera
to C. pyloridis, we found that most of the C. pyloridis strains
share core antigens (Fig. 8), and immunoblotting with anti-
serum to *Salmonella* lipid A (29) confirmed that lipid A is a
common constituent of the LPS of C. pyloridis (data not shown).

However, *C. pyloridis* O antisera showed four different
patterns of recognition of the LPS antigens (Fig. 8). The
response of several O antisera to LPS from *C. pyloridis*
84-182 was essentially identical to their response to LPS
from *C. pyloridis* 85-456 (data not shown). All of the O
antisera tested recognized the core region except the O
antisera directed toward *C. pyloridis* 84-183 (lane e); the
same results were observed with OH antiserum toward this
strain. The reactions of O antisera to *C. pyloridis* 86-86 and
84-183 were similar (data not shown), with a clear recogni-
tion of several high-molecular-weight bands by the O (lane e)
and OH (lane h) antisera raised against strain 84-183 and
recognition of the core region by O antisera against *C.
pyloridis* 84-181 (lane c) and 85-456 (lane f). *C. pyloridis*
84-181 OH antiserum (lane g) also recognized the core
region. Finally, LPS antigens from strains 84-180 and 86-63
were recognized by O antisera against *C. pyloridis* 84-180
(lane b) and 84-181 (lane c) in the core region. However,
immunoblots of *C. pyloridis* 84-180 LPS showed some high-
molecular-weight complexes with the homologous serum,
whereas blots of *C. pyloridis* 86-63 LPS did not. *C. pyloridis*
86-63 LPS had three high-molecular-weight bands recogni-
tized by the O antiserum to 84-181 (lane c) which were not
observed when O antiserum to *C. pyloridis* 84-180 (lane b)
was used. Although both antigens were recognized by O
antisera to *C. pyloridis* 84-182, LPS from *C. pyloridis*
84-180 showed a much stronger reaction, indicating that the
antisera differ from one another and from those of other
strains.

**DISCUSSION**

Currently there is much interest in *C. pyloridis* and other
gastric *Campylobacter*-like organisms (30) because of their
frequent association with inflammatory gastroduodenal condi-
tions (15; J. Warren, Letter, Lancet i:1273, 1983). In this
report, we have been concerned with the antigenic charac-

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**FIG. 6.** Immunoblots of rabbit sera against whole cells of *C. pyloridis* 84-182, 84-180, 85-456, and 86-63 which represent four different
patterns. Sera were obtained from rabbits that were unimmunized (lanes a); immunized with Formalin-treated cells (OH serum) of *C. pyloridis*
84-181 (lanes b), 84-182 (lanes c), 84-183 (lanes d), and 86-63 (lanes e); immunized with heat-treated whole cells (O serum) of *C. pyloridis*
84-182 (lane f for *C. pyloridis* 84-182), 84-180 (lane f for *C. pyloridis* 84-180), 85-456 (lane f for *C. pyloridis* 85-456), or 84-181 (lane f for *C.
pyloridis* 86-63); or immunized with Formalin-treated cells (OH serum) of *C. fetus* 82-40 (lanes g) or *C. jejuni* 79-193 (lanes h). All sera
were diluted 1:100, and horseradish peroxidase conjugated to swine anti-rabbit immunoglobulins was used at a dilution of 1:1,500. The numbers
on the left indicate molecular weight in thousands. The arrow indicates a common band at 62,000 which corresponds to flagellar antigen.
The SDS-PAGE profiles of acid-extracted material from the five C. pyloridis strains studied indicates conservation of these cell surface molecules. The common bands which migrate at 62,000, 44,000, and 29,000 are similar to those seen in C. jejuni (2) and may represent common structures whose function is conserved at the genus or subgenus level. The 62,000-molecular-weight protein which represents a major surface structure on C. pyloridis is recognized by antiserum directed against purified C. jejuni flagellae (4) and probably represents a flagellar protein of C. pyloridis.

Our study on outer-membrane preparations showed that C. pyloridis isolates can be differentiated from one another on the basis of their SDS-PAGE profiles and that these are distinct from those of C. fetus, C. faecalis, C. jejuni, and C. coli (3). The observation that C. pyloridis outer-membrane profiles have more variability between strains than do whole-cell or acid-extractable preparations suggests that the outer-membrane constituents are less highly conserved. This property could be useful for typing organisms as has been done for Neisseria meningitidis (20), N. gonorrhoeae (5), and Haemophilus influenzae (1, 6).

As expected for gram-negative organisms, LPS are present in C. pyloridis, and stability appears to be independent of the number of in vitro passages. The semirough characteristics of the LPS of some C. pyloridis strains, migrating in a manner similar to that of the Ra chemotype of Salmonella, resemble the PAGE profiles of the LPS from several other bacterial genera, including Neisseria (19, 23), Bacteroides (31), and Pasteurella (13), and also C. jejuni (27). Although the LPS of these C. pyloridis isolates appear to lack the several repeating saccharide units responsible for serospecificity among several genera of the Enterobacteriaceae family (12), these molecules might contain, within a relatively small structure, both serospecific and nonserospecific antigens as was observed for C. jejuni (29). The results of heterologous immunoblotting studies (Fig. 8) support this hypothesis.

LPS from several of the C. pyloridis strains resembled those of smooth-type Enterobacteriaceae with a typical washboard pattern, but in some cases fewer complexes were present. Moreover, the profiles of C. pyloridis 84-183 resembled that of C. fetus LPS with a classical washboard pattern but without complexes migrating at molecular weights below 25,000 (28); different from C. fetus is that the core region could be easily visualized in C. pyloridis. The heterogeneity observed in LPS profiles of C. pyloridis could be used to develop a serotyping system based on heat-stable antigens as has been done for C. jejuni (26). However, it also is possible that LPS type correlates with pathogenicity as is true for E. coli (10) or Salmonella sp. (11). Although the presence of C. pyloridis on the gastric epithelium is strongly associated with gastritis (Walters et al., Letter), the etiologic role of the organisms is not certain (R. A. Burnett, J. A. H. Forrest, R. W. A. Girdwood, and C. R. Fricker, Letter, Lancet i:1349, 1984). It is possible that, as with E. coli strains, pathogenicity is related to only certain serotypes. More work is needed to answer this question.

Immunoblotting of whole cells with sera from rabbits immunized with C. pyloridis cells demonstrated the presence of both common and specific antigens among the seven strains of C. pyloridis. In studies of seven strains, were were able to recognize four distinct antigenic profiles. The two strong areas of recognition in the regions between molecular weights 45,000 and 66,000 and below 31,000 correspond to the regions in which most of the major bands were resolved in whole-cell, outer-membrane, and acid-extracted preparations in SDS-PAGE.

Results of our previous studies showed that Campylobacter species possess a core LPS structure (27, 28). By immunoblotting, we found that most of the C. pyloridis strains share core antigens with one another. That facts that the core LPS regions of C. pyloridis are antigenically related and lipid A antigenicity is shared (21) favor the hypothesis that the widely shared cross-reactivities observed among LPS from gram-negative organisms are due in part to antigenic similarities among the lipid A regions (29).

The results obtained by immunoblotting of LPS preparations from six strains showed four different patterns. However, the distribution of the strains into these patterns is not the same as the distribution based on the whole-cell (protein) patterns. It is likely (i) that, as with C. jejuni (24), there exists considerable antigenic diversity among C. pyloridis strains with independent strain-specific LPS (O) and protein antigens or (ii) that more than one species included in the C. pyloridis group exists. This is not surprising for organisms
that live in mammalian hosts. This paper represents a study of the major antigens of C. pyloridis and is only the first step toward a serotyping system. Conversely, conservation of several protein antigens among all of the strains tested suggests that there also exist group antigens, as we observed with the ELISA, which may be useful for development of serologic assays of C. pyloridis infection or for eventual vaccine development.

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LITERATURE CITED


FIG. 8. Immunoblot of proteinase K-treated whole-cell lysate preparations of C. pyloridis 84-182, 86-86, 84-180, and 86-63 against rabbit serum. Sera were from rabbits that were unimmunized (lanes a); immunized with boiled whole cells (O serum) of C. pyloridis 84-180 (lanes b), 84-181 (lanes c), 84-182 (lanes d), 84-183 (lanes e), or 85-456 (lanes f); or immunized with Formalin-treated cells (OH serum) of C. pyloridis 84-181 (lanes g) or 84-183 (lanes h). All sera were diluted 1:100.


