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Antigenic Variation of *Campylobacter* Flagella

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Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of flagella dissociated from strains of *Campylobacter coli* and *Campylobacter jejuni* belonging to the heat-labile serogroup LIO 8 showed that some strains were capable of producing flagellin subunits of two different molecular weights (M_rs), 59,500 and 61,500. Immunoelectron microscopy of cultures of the type strain of this serogroup, *C. coli* VC167, showed the presence of two flagellum filaments of different antigenic specificity. Epitopes on the surface of one of these flagella bound antibodies in LIO 8 typing antiserum, and Western blotting (immunoblotting) and immunoprecipitation showed that the flagellum was composed of flagellin of M_r 61,500. The other flagellum antigenic type did not bind LIO 8 antibodies but did possess serospecific epitopes which bound a second polyclonal antiserum, LAH2. This second antigenic flagellum type was composed of the M_r 59,500 flagellin. Cells producing either of the flagellum antigenic types serotyped as LIO 8, indicating that flagella composed of the M_r 61,500 flagellin do not carry the serological determinants for this serogroup. The ability of *C. coli* VC167 to produce these flagella of different subunit M_rs was shown to represent a bidirectional antigenic variation. When measured in culture medium, the phase 1-to-phase 2 transition occurred at a rate of approximately 2.0 $\times 10^{-5}$ per cell per generation, and the phase 2-to-phase 1 transition occurred at a rate of 1.2 $\times 10^{-6}$ per cell per generation.

The thermophilic campylobacters, *Campylobacter jejuni* and *Campylobacter coli*, are gram-negative, spiral bacteria which rank among the leading causes of bacterial diarrhea in humans throughout the world (3, 5, 25, 28). As a consequence, these organisms are the subject of many epidemiological investigations. Of the various typing methodologies that have been used by various workers, serological typing remains the method of choice. One widely used typing system is that developed by Lior et al. (12). The Lior scheme is based on thermolabile antigens and involves slide agglutination of live bacteria with serotype-specific polyclonal antisera. The scheme has some 62 serotypes for the thermophilic campylobacters, including 39 for *C. jejuni* and 18 for *C. coli* (13).

Campylobacters are actively motile by means of their polar flagella, and this motility allows them to colonize the mucus lining the intestinal tract (11). Once established in the mucus, campylobacters are ideally situated to produce gastrointestinal disease. Motility appears to be a major determinant of *Campylobacter* pathogenicity, since nonmotile mutants and variants appear to be unable to colonize the gastrointestinal tract of experimental animals or human volunteers (19, 28). In addition to this role in mucus colonization, the flagellum is a major antigen of the campylobacter cell (14, 15, 20, 29) and, in several LIO serogroups, appears to carry the serogroup-specific epitopes, since nonflagellated mutants lose their capacity to be serotyped by the Lior procedure (29, 30). For most LIO serogroups however, the contribution of flagellum epitopes to serotypic specificity has yet to be determined.

In this study we examined the contribution of flagellum epitopes to the serospecificity of the LIO 8 serogroup. This thermolabile serogroup contains strains of both *C. coli* and *C. jejuni* and is one of the more common serogroups isolated from all sources (12, 13). Indeed it is the serogroup of *C. coli* most commonly isolated from human sources (13), and the type strain of the serogroup is a *C. coli*. We found that the flagella on strains of both *C. jejuni* and *C. coli* belonging to the LIO 8 serogroup, including the serogroup type strain, were subject to antigenic variation, and here we report our findings.

MATERIALS AND METHODS

Bacterial strains. The *Campylobacter* strains examined in this study all belonged to serogroup LIO 8 and were graciously provided by H. Lior, Enteric Reference Laboratory, Laboratory Center for Disease Control, Ottawa, Ontario, Canada. *C. coli* VC143, VC145, VC146, VC147, and VC149 were isolated from human feces in Bangladesh, whereas *C. coli* VC167 was isolated from human feces in the United Kingdom. *C. jejuni* VC150, VC154, VC155, VC156, VC159, and VC163 were isolated from human feces in the United Kingdom. The serologically unrelated control strain *C. jejuni* VC74 was from this laboratory and was isolated from human feces. Stock cultures were maintained at −70°C in 15% (vol/vol) glycerol–Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). Cultures were grown at 37°C in anaerobic jars on chocolate-blood agar plates. An atmosphere containing 5% oxygen and 10% carbon dioxide was produced with a gas-generating kit for campylobacters (Oxoid Ltd., Basingstoke, United Kingdom).

Electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with a mini-slab gel apparatus (Hoeffer Scientific Instruments, San Francisco, Calif.) by the method of Laemmli (10). Protein solubilized in sample buffer was stacked in 4.5% acrylamide (100 V, constant voltage) and separated with 7.5% acrylamide (200 V, constant voltage). On occasion, separating gels of 12.5 and 15% were used. Protein was stained with Coomassie blue or by the silver staining procedure of Wray et al. (31).

Separated proteins were transferred to nitrocellulose by

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the methanol-tris-glycine system of Towbin et al. (27) as previously described (14, 15).

Glycine extraction of flagellin. Small-scale rapid extraction of flagellin was achieved by acid pH dissociation of flagellum filaments (14). A 0.3-cm loopful of bacteria, or a single colony was suspended in 100 μl of 0.2 M glycine-hydrochloride (pH 2.2) and allowed to sit at room temperature for 5 min. Bacteria were then removed by centrifugation at 12,000 × *g* for 3 min.

Purification of flagellin. Flagella were isolated from 24-h cultures by homogenization, and flagellin was partially purified by the sequential acid pH dissociation-ultracentrifugation-neutral pH association procedure previously described (14, 15). Flagellin was purified to homogeneity, as assessed by a single polypeptide band on a silver-stained SDS-PAGE gel, and by N-terminal amino acid sequence analysis by high-performance liquid chromatography with a reverse-phase RP-8 Spheri-10 column (Brownlee Laboratories, Santa Clara, Calif.) and a Beckman high-performance liquid chromatography system (Beckman Instruments Inc., Berkeley, Calif.). Solvent A was 0.1% trifluoracetic acid in water, solvent B was 100% acetonitrile. The flow rate was 1 ml/min, and chromatography was performed at room temperature. Elution of flagellin at 50% acetonitrile was achieved by using a discontinuous gradient. In the case of phase 2 flagellin, partially purified flagellin was subjected to ion-exchange chromatography with a mono-Q column (Pharmacia Fine Chemicals, Uppsala, Sweden). The column was run isocratically for 10 min in 10 mM Tris (pH 4.0), and a gradient from 0 to 0.1 M NaCl in 10 mM Tris (pH 4.0) was then applied to the column for 5 min. The column was run isocratically until the flagellin was eluted.

Selection of cells producing flagella of different antigenic specificity. Cultures were grown for 24 h and then streaked on one side of a chocolate-blood agar plate from which a center strip of agar had been removed. A sterile filter paper strip was placed across the center well to form a bridge, and 25 μl of a 1:20 dilution of antiserum containing antibodies to either phase 1 or phase 2 flagella was applied to the filter paper. When growth appeared on the other side of the bridge, cells were collected, glycine extracts were prepared, and the flagellin M_r was determined by SDS-PAGE (7.5% polyacrylamide).

Rate of flagellum antigenic variation. The thioglycolate medium of Caldwell et al. (4) was used to determine the rate of flagellum antigenic variation. Warm thioglycolate medium (BBL) containing 0.33% (wt/vol) agar was prepared. After growth for approximately 30 generations, bacteria from a single colony producing phase 1 or phase 2 flagella were added at an appropriate density to give between 200 and 500 colonies per plate. A 1:500 dilution of antiserum to phase 1 (antiserum LAH1) or phase 2 (antiserum LAH2) flagella was then added, and the plates were poured. Controls with no added antiserum were also poured. After 2 days the rate of flagellum antigen alteration was determined by counting the number of motile and nonmotile colonies in the antiserum-containing agar. The M_r of flagellin from nonmotile and motile colonies was determined by SDS-PAGE of glycine extracts.

Antibodies. Polyclonal antiserum SML2 and monoclonal antibody 39 (as ascites, immunoglobulin G2a) to cross-reactive, non-surface-exposed *Campylobacter* flagellin epitopes were prepared as previously described (15). Antiserum containing antibodies to native flagellum filaments on *C. coli* VC167 was prepared by immunizing adult New Zealand White rabbits by the method of Penner and Hennessy (22) with a saline suspension (A_{625}, 0.375) of live bacteria producing either phase 1 (antiserum LAH1) or phase 2 (antiserum LAH2) flagella. Rabbits were injected intravenously five times in a 2-week period with 1, 2, 2, 4, and 4 ml of the suspension. The serum was collected on day 48. Antiserum LAH3 directed against purified phase 1 flagellin was also prepared. Flagellin (600 μg) emulsified with Freund complete adjuvant (900 μl) was injected subcutaneously. A booster injection of flagellin with Freund incomplete adjuvant was given after 21 days, and the sera were collected on day 35. When required, antibodies to heat-stable antigens were removed by twice absorbing antisera with a homologous heat-stable antigen suspension as previously described (14). Both antiserum LAH1 and antiserum LAH3 were absorbed with boiled phase 1 cells in this way and with live cells bearing phase 2 flagella to remove any antibodies which might cross-react with phase 2 flagella. Antiserum was stored at −20°C. Prebleed serum was taken and used as the control serum.

Immunoelectron microscopy. Bacterial cells on Formvar carbon-coated grids were incubated with antiserum in a humid chamber at 37°C for 30 min. After three washes in distilled water the cells were negatively stained with 1% (wt/vol) uranyl acetate (pH 4.2) and examined in a Phillips EM 300 electron microscope.

Indirect fluorescent antibody testing. Indirect fluorescent antibody testing of living cells was performed as previously described (15).

Slide agglutination. The ability of antisera to agglutinate live *Campylobacter* cells was tested by the method of Lior et al. (12) at the 1:16 dilution recommended for the batch of antiserum used. The presence or absence of agglutination was determined after 1 min.

ELISA. The enzyme-linked immunosorbent assay (ELISA) was done essentially by the method of Engvall and Perlmann (7). Antigen was tested, in triplicate, at 0.5 to 2 μg per well, and the developing antibody was alkaline phosphatase-conjugated goat anti-mouse or anti-rabbit immunoglobulin (Sigma Chemical Co., St. Louis, Mo.). The A_{405} was read with an EIA model 310 ELISA reader (Biotek Instruments Inc., Highland Park, Vt.). Bovine serum albumin was used as an antigen control.

Radioimmunoprecipitation. Flagellum protein in glycine extracts or as purified flagellin was ^{125}I radiolabeled by a modified Hunter and Greenwood chloramine T procedure (8) as described previously (14). For immunoprecipitation the radiolabeled sample was diluted fivefold in buffer [0.15 M NaCl, 0.5% Nonidet P-40, 0.1% SDS, 20 mM Tris (pH 7.8), 1 mM EDTA, 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], and 100 μl of the sample was placed in a conical tube. Antibody (25 μl at a predetermined dilution) was added and left for 15 min at room temperature. Protein A–Sepharose 4B (50 μl of a 50% suspension) was then added and left for a further 15 min. After dilution to 10 ml, the protein A-antibody complexes were removed by centrifugation and washed twice. The pellet was suspended in 0.2 ml of buffer and placed in a microcolumn. The microcolumn was dried by centrifugation, 20 μl of SDS sample buffer was added, and the column was heated to 95°C for 5 min. Solubilized protein was eluted from the column by centrifugation, and the sample was analyzed by SDS-PAGE followed by autoradiography.

Western blotting (immunoblotting). After electroblotting, antigenic polypeptides were detected by reaction with antiserum followed by incubation with ^{125}I-radiolabeled *Staphylococcus aureus* protein A as previously described (14).

Detection of bound, radiolabeled protein A was accomplished by autoradiography of washed and dried nitrocellulose sheets.

RESULTS

Flagellum antigenic types. Initial investigation of the surface antigens of strains belonging to *Campylobacter* thermolabile serogroup LIO 8 employed indirect fluorescent antibody testing analysis with LIO 8 typing antiserum. This antiserum had been produced in rabbits against formalinized cells of *C. coli* VC167, and antibodies to homologous heat-stable antigen had been removed by absorption (12). When live cells of this LIO 8 type strain VC167 were stained at a 1:500 dilution of antiserum, LIO 8 antibodies were seen to bind to the flagella of this strain but not to the cell surface. This was also the case when live cells of two other LIO 8 strains, *C. jejuni* VC156 and *C. jejuni* VC159, were examined by this technique. This indicated that the flagellum filament was a major antigen on these LIO 8 strains; since LIO 8 antibodies did not bind to the flagella of serologically unrelated *C. jejuni* VC74 (serogroup LIO 11) or strains belonging to several other LIO serogroups in control indirect fluorescent antibody testing assays, flagella on these three LIO 8 strains appeared to exhibit antigenic specificity.

To identify the flagellum subunit protein binding the antibodies contained in the LIO 8 typing antiserum, the flagellum proteins of a number of strains belonging to serogroup LIO 8 were extracted from motile cells by the 0.2 M glycine buffer (pH 2.2) procedure and analyzed by SDS-PAGE. Surprisingly, the flagellin extracted from some of the LIO 8 strains in this experiment displayed a subunit M_r of 59,500 (Fig. 1A, lanes 1 through 5 and 7), whereas the flagellin from other strains displayed a subunit M_r of 61,500 (lanes 9 and 12). In several strains both flagellin subunits were present (lanes 6, 8, 10, and 11). This was also the case with some cultures of LIO 8 type strain *C. coli* VC167 (data not shown). When randomly selected sequential subcultures of representative LIO 8 strains were subsequently analyzed for the production of different subunit M_r flagellins, strains VC146, VC154, VC156, VC164, and VC167 were all capable of producing both flagellins. Changes in the M_r of the predominant flagellin subunit being produced by a given strain were also seen occasionally, albeit not reproducibly. In the case of strains VC143, VC144, VC147, and VC149, only the M_r 59,500 flagellin was ever seen with SDS-PAGE. However, regardless of the M_r of the flagellin being produced, slide agglutination tests at the 1:16 dilution recommended for the LIO 8 typing antiserum confirmed that all of the strains belonged to serogroup LIO 8.

Immunoelectron microscopic examination of cultures of *C. coli* VC167 producing two flagellins with different M_rs after reaction with LIO 8 typing antiserum revealed the presence of flagellum filaments with different apparent antigenic specificities. In one case the LIO 8 antibodies completely decorated the surface of the flagellum filament, whereas the other flagellum type did not bind LIO 8 antibodies (Fig. 1B). Two antigenic types of flagellum filaments were also seen in cultures of VC156 which produced two flagellin subunits. In both cases, however, single cells exhibited only a single flagellum antigenic type. To determine which of the flagellin subunits was reacting with the LIO 8 antiserum, radioimmunoprecipitation experiments were performed. When glycine extracts of cultures of VC167, VC156, and VC159 were immunoprecipitated with a 1:100 dilution of LIO 8 antiserum, the M_r 61,500 flagellin was the only

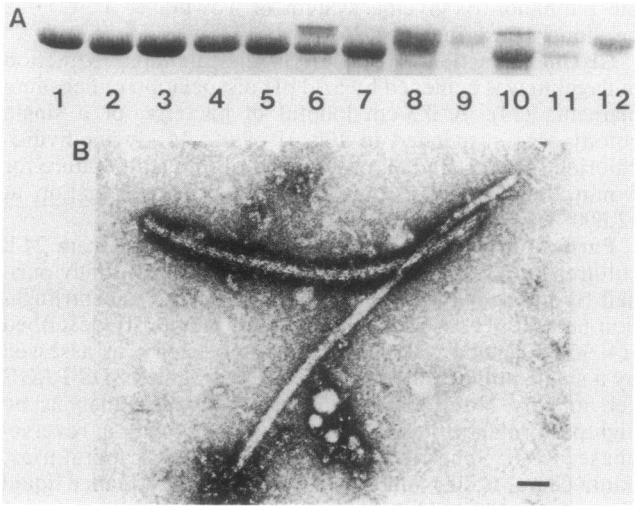

FIG. 1. (A) SDS-PAGE (7.5% polyacrylamide) of glycine-extracted flagellins of *C. jejuni* (lanes 1 through 5) and *C. coli* (lanes 6 through 12) strains belonging to serogroup LIO 8 stained by Coomassie blue. Lanes: 1, VC143; 2, VC147; 3, VC146; 4, VC145; 5, VC149; 6, VC150; 7, VC154; 8, VC155; 9, VC156; 10, VC159; 11, VC162; 12, VC163. (B) Immunoelectron microscopy of flagella from a culture of *C. coli* VC167 reacted with polyclonal LIO 8 antiserum and negatively stained with 1% uranyl acetate (pH 4.2), showing two flagellum filaments with different antigenic specificity. One flagellum filament has been completely coated with antibodies, whereas the second filament has not bound antibody. Bar, 50 nm.

polypeptide to be precipitated (data not shown). No flagellin was immunoprecipitated by the LIO 8 antiserum in control assays with glycine extracts of serologically unrelated *C. jejuni* VC74 (LIO 11) or strains belonging to two other LIO serogroups (13 and 28) tested as controls. Moreover, no flagellin was immunoprecipitated from glycine extracts of VC143 which contained the M_r 59,500 flagellin. Since slide agglutination confirmed that VC143 belonged to serogroup LIO 8, this indicated that the epitopes determining the serospecificity of this particular serogroup were not carried on the M_r 61,500 flagellin-containing flagella.

Demonstration of antigenic variation. One explanation for these observations was that in those strains where two different flagellins were produced, the *Campylobacter* cultures being glycine extracted contained one population of cells producing flagella composed of flagellin monomers of one M_r and antigenic type and another population producing flagella containing flagellin monomers of different M_r and different antigenic type, and that the production of these different flagellins was subject to switching. The strategy selected to test whether *Campylobacter* flagellin production was indeed subject to such antigenic variation was to use antibody-mediated immobilization to select cells producing flagella with different antigenicity. Such cells would retain their motility in the presence of the LIO 8 antiserum. The technique employed a sterile filter paper bridge containing 25 μl of a 1:20 dilution of LIO 8 antiserum to connect two separated culture medium surfaces. The medium on one side of the bridge was then inoculated with cells from a single colony of campylobacters; after incubation for 48 h, bacteria which had crossed the bridge were isolated by subculture.

Figure 2A (lanes 2 and 3) shows the results of an experiment in which a clone of LIO 8 type strain VC167 produced M_r 61,500 flagellin before bridge selection (lane 2) but, after five bridge transfers, produced flagellin of M_r 59,500 (lane 3).

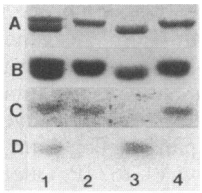

FIG. 2. Antigenic variation of flagellins produced by *C. coli* VC167. Lanes: 1, mixture of purified phase 1 and phase 2 flagellins; 2, purified phase 1 flagellin; 3, purified phase 2 flagellin; 4, flagellin purified from a *C. coli* VC167 culture switched back from the production of phase 2 flagella to the production of phase 1 flagella. (A) SDS-PAGE (7.5% polyacrylamide) stained by Coomassie blue showing differing subunit M_rs for phase 1 (61,500) and phase 2 (59,500) flagellins: (B) autoradiogram of Western blot with a 1:10,000 dilution of monoclonal antibody 39 to *Campylobacter* flagellin; (C) autoradiogram of Western blot with a 1:100 dilution of polyclonal antiserum LIO 8; (D) autoradiogram of Western blot of purified flagellins with a 1:100 dilution of polyclonal antiserum LAH2.

Confirmation that the M_r 59,500 protein was flagellin was provided by its ability to react in Western blot assays with monoclonal antibody 39 to *Campylobacter* flagellin (Fig. 2B, lanes 1 and 3) and with polyclonal anti-*Campylobacter* flagellin antiserum SML2 (data not shown). Cells producing either flagellin serotyped as LIO 8 by slide agglutination. However the M_r 59,500 flagellin clearly exhibited altered antigenic specificity with respect to the epitopes recognized by the LIO 8 typing antiserum, since it did not react with the LIO 8 typing antiserum in Western blots (Fig. 2C, lanes 1 and 3) and was not immunoprecipitated by this antiserum (data not shown). This switching of flagellin antigenicity was repeated on four separate occasions with VC167 and the LIO 8 typing antiserum (including a fresh culture of the LIO 8 type strain supplied by H. Lior) and was also accomplished with two other LIO 8 strains, VC156 and VC159 (data not shown). The switching of strain VC167 from the production of antigenic phase 1 flagellin (M_r 61,500, LIO 8 positive) to the production of antigenic phase 2 flagellin (M_r 59,500, LIO 8 negative) was also confirmed with two additional antisera prepared in this laboratory to phase 1 flagella of strain VC167 (antisera LAH1 and LAH3).

Antiserum LAH2 was then produced against live phase 2 cells of VC167 and was used in a bridge experiment to select back VC167 cells with flagella in antigen phase 1 (Fig. 2A, lane 4). In Western blot experiments this phase 2 antiserum was able to react specifically with phase 2 VC167 flagellin (Fig. 2D, lanes 1 and 3) but not with phase 1 VC167 flagellin (Fig. 2D, lanes 1, 2, and 4). Control assays with bridge selection in the absence of antiserum failed to provide cells with an altered flagellum phase.

ELISA. An ELISA was used to provide a measure of the structural similarities and differences of the M_r 61,500 antigenic phase 1 and M_r 59,500 antigenic phase 2 flagellins. The two flagellin monomers were shown to be highly related with respect to the non-surface-exposed regions of the molecules recognized by polyclonal antiserum SML2, as demonstrated by the virtually identical ELISA curves obtained with this antiserum (Fig. 3A). The structures of the two flagellins were however measurably different with respect to the epitopes recognized by antibodies in the LIO 8 typing antiserum (Fig. 3B) and were also measurably different with respect to the epitopes recognized by antibodies in antiserum LAH2 (Fig. 3C).

Frequency of antigenic variation. The pour plate method of Caldwell et al. (4) was then used to estimate the rate of antigenic variation of *C. coli* VC167 flagella by determining colonial motility in thioglycolate motility medium containing a either phase 1 or phase 2 antiserum. The M_r of the flagellins produced by all colonies whose motility was inhibited by the presence of antiserum, as well as that of 50 motile colonies chosen at random, was determined by SDS-PAGE after glycine extraction of the *Campylobacter* cells. In all cases flagellin of the appropriate M_r for the antibody being used was shown, and no nonflagellated colonies were detected in test or control plates. With this technique, the rate of the phase 1-to-phase 2 switch in *C. coli* VC167 was approximately 2.0×10^{-5} per cell per generation, whereas the phase 2-to-phase 1 switch rate was approximately 1.2×10^{-6} per cell per generation (Table 1).

DISCUSSION

This study has shown that the flagella of certain strains of *C. coli* and *C. jejuni* undergo antigenic variation. In the case of the serogroup LIO 8 type strain studied here, *C. coli* VC167, this antigenic variation was reversible and corresponded to the production of flagellum filaments containing flagellin monomers of subunit M_r 61,500 in antigen phase 1 and flagellum filaments containing flagellin of subunit M_r 59,500 in antigen phase 2. The two flagellin subunits were shown to be antigenically related with respect to regions of the monomer which are not exposed on the surface of the native flagellum filament (15). This is consistent with the structural similarities shared by other *Campylobacter* flagellins (15). The two flagellins were however antigenically distinct with respect to other epitopes. In the case of the phase 1 flagella, these serospecific epitopes were recognized by antibodies present in LIO 8 typing antiserum. Significantly, however, whereas the LIO 8 antiserum contained a high titer of these antibodies which bound to the antigenic phase 1 flagellum filaments, these flagella did not confer LIO 8 serogroup specificity, since *C. coli* VC167 cells producing phase 2 flagella also typed as LIO 8. The surface component conferring LIO 8 serogroup specificity has yet to be identified.

Several other strains belonging to the serogroup LIO 8 were also shown to be capable of producing flagellins of

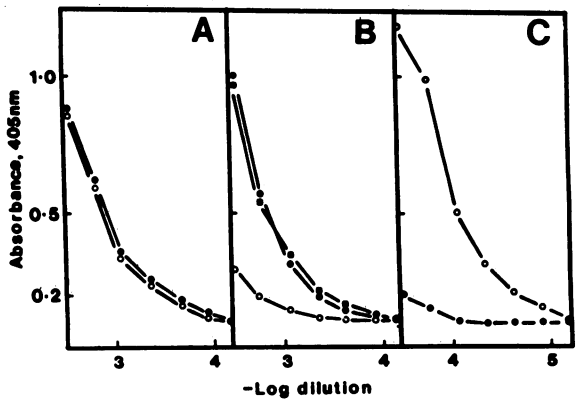

FIG. 3. ELISA of purified *C. coli* VC167 phase 1 (●) and phase 2 (○) flagellins. (A) Flagellin (1 μg) reacted with polyclonal antiserum SML2 to non-surface-exposed, cross-reactive *Campylobacter* flagellin epitopes (15); (B) 2 μg of flagellin reacted with polyclonal LIO 8 typing antiserum (■, flagellin purified from a *C. coli* VC167 culture switched back from the production of phase 2 flagella to the production of phase 1 flagella); (C) 1 μg of flagellin reacted with LAH2 antiserum.

TABLE 1. Variation of flagella antigenicity in *C. coli* VC167

Variation	No. of variants	Total no. of cells examined[a]	Variation rate[b]
Phase 1 to 2	19	29,944	2.0×10^{-5}
Phase 2 to 1	3	64,210	1.2×10^{-6}

[a] The numbers for the transitions are the totals from three separate experiments.

[b] The ratio of the number of variants to the total number of cells plated gave the fraction of cells which had undergone transition. This ratio was divided by the approximate number of generations the population had undergone to yield the transition rate (6, 23, 26).

different M_r, and flagellum production by these strains also appeared to be subject to antigenic phase variation. Other studies (Logan and Trust, unpublished data) have shown that this ability to produce flagellins of different subunit M_r, and presumably to produce antigenically distinct flagellum filaments, is not restricted to the LIO 8 strains examined here. In our hands, strains which produce flagellins of different subunit M_r have been shown in LIO serogroups 2, 11, and 13. One example is the LIO 11 control strain used in this study, *C. jejuni* VC74. Examination of published data of others also shows the presence of multiple flagellins in a variety of strains of both *C. jejuni* and *C. coli* (1, 20, 21). Indeed, antigenic variation of flagella likely explains the observations of Newell (20), who reported that some anti-flagellum monoclonal antibodies raised to purified flagella from *C. jejuni* 81116 (LIO 6) reacted with an M_r 61,500 flagellum protein, whereas others reacted with a protein of approximate M_r 60,000, which the author could not identify. Our findings suggest that this M_r 60,000 protein of *C. jejuni* 81116 is likely the antigenic phase 2 flagellin of this strain. Flagellum antigenic variation may also extend to *Campylobacter fetus*, since McCoy et al. (16) reported the presence of two flagellum-containing bands during their cesium chloride purification of sheared *C. fetus* flagella and also observed differences both in flagellum filament diameter and flagellum internal structure at different phases of *C. fetus* growth.

In addition to this ability of some *Campylobacter* strains to alternately express antigenically different flagella, Caldwell et al. (4) have reported that some strains appear to undergo a bidirectional transition between flagellated (Fla+) and nonflagellated (Fla^-) phenotypes. One of the strains exhibiting this on-off switching was *C. jejuni* 81116 (discussed above). We did not observe the Fla^+-to-Fla^- transition in *C. coli* VC167, and all nonmotile colonies in antibody-containing media in frequency determination experiments were shown to be producing flagella of the appropriate M_r. Using this technique, the frequency we estimated for the flagellum antigenic phase 1-to-phase 2 switch in VC167 was 2.0×10^{-5} per cell per generation, whereas the phase 2-to-phase 1 transition was 1.2×10^{-6} per cell per generation. For technical reasons we believe these to be conservative estimates of the frequency of flagellum transition of this strain. These rates are however consistent with those of *Salmonella* flagellum phase variation, in which the phase changes are on the order of 10^{-3} to 10^{-5} per cell per generation for some strains of *Salmonella typhimurium* but may be as low as 10^{-7} for strains of *Salmonella abortus-equi* (9, 26).

In the case of flagellum antigenic phase variation by *Salmonella* species, as with other examples of surface antigen phase variation, a programmed DNA rearrangement is involved (2, 6, 17, 18). In *S. typhimurium* the rearrangement involves the reversible inversion of a 995-base-pair sequence 16 base pairs upstream from the gene which specifies H2 flagellin synthesis (24). The molecular mechanism responsible for the flagellum antigen variation in *Campylobacter* species is unclear at this time. However, a reversible DNA rearrangement accompanying flagellum antigen variation has been detected in *C. coli* VC167 (P. Guerry, E. C. Lee, D. H. Burr, O. R. Majam, L. A. Harris, S. M. Logan, and T. J. Trust, Abstr. Ann. Meet. Am. Soc. Microbiol. 1986, D75, p. 78). A cause-effect relationship between this rearrangement and the antigenic phase transition is now under investigation.

The role of flagella antigenic variation in the pathogenesis of *Campylobacter* infection now needs to be investigated. Certainly in a number of other cases the programmed gene rearrangements which affect the expression of surface antigens are thought to confer a pathogenic advantage (6, 17, 18). The ability to produce antigenically different flagella is also likely to have major ramifications for the seroepidemiology of *Campylobacter* infections, for although the flagellum switching studied here did not affect the serotyping results in the case of the LIO 8 strains tested, typing of strains belonging to LIO serogroups 5, 6, 7, and 17 (29, 30) where flagella appear to carry the serogroup determinants would obviously be affected by antigenic variation. Certainly serotyping systems for *Campylobacter* species which are based on protein antigens will need to be expanded to include flagella in different antigenic phases. Furthermore, since DNA rearrangements occur in *Campylobacter* species, the possibility of phase variation of thermostable antigens cannot be ruled out. Indeed, it may become necessary to treat *Campylobacter* species serotyping like that for *Salmonella* species and determine both the thermostable and thermolabile antigens of isolates.

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