INFECTION AND IMMUNITY, Dec. 1985, p. 941–943
0019-9567/85/120941-03$02.00/0

Vol. 50, No. 3

Reversible Expression of Flagella in *Campylobacter jejuni*

M. B. CALDWELL,* P. GUERRY, E. C. LEE, J. P. BURANS, AND R. I. WALKER

Infectious Diseases Program Center, Naval Medical Research Institute, Bethesda, Maryland 20814

Received 28 June 1985/Accepted 26 August 1985

***Campylobacter jejuni* 81116 and A3249 undergo a bidirectional transition between flagellated (Fla^+) and aflagellated (Fla^-) phenotypes. When measured in culture medium, the $Fla^+ \rightarrow Fla^-$ transition occurred at a rate of 3.1×10^{-3} to 5.9×10^{-3} per cell per generation, and the Fla^- to Fla^+ transition occurred at a rate of 4.0×10^{-7} to 8.0×10^{-7} per cell per generation. However, passage through a rabbit intestine markedly favored the Fla^+ phenotype.**

Campylobacter jejuni is a major causative agent of acute bacterial enteritis in humans. Unlike most enteropathogens, no system (i.e., transformation, transduction, conjugal mobilization) for transfer of chromosomal genes has been reported for *C. jejuni*. Similarly, although conjugative R plasmids have been described, transfer is restricted to the genus *Campylobacter* (21). The absence of a genetic system has greatly hampered study of the virulence determinants of this organism. *C. jejuni* has been reported as being invasive (13, 16) and enterotoxigenic (7, 10, 17), properties which could elicit the characteristic symptomatology associated with the disease. In addition, the flagella of *C. jejuni* have been implicated as virulence determinants. Several studies have compared the virulence of flagellated (Fla^+) strains with spontaneously isolated aflagellated (Fla^-) variants. Newell et al. (12, 13) compared the ability of strains 81116 Fla^+ and 81116 Fla^- to colonize the gastrointestinal tract of infant mice and found that the Fla^- variant colonized much less effectively than the Fla^+ parent. In addition, R. Black and co-workers (personal communication) fed human volunteers a mixture of strains A3249 Fla^+ and A3249 Fla^- and were able to isolate only flagellated forms from subsequent stool cultures of the infected individuals. The present study was initially conceived to compare the virulence of the Fla^+ and Fla^- variants of these strains in rabbits by using the RITARD (removable intestinal tie-adult rabbit diarrhea) procedure (3, 19). During these studies, we observed that the Fla^+ and Fla^- phenotypes of 81116 and A3249 are very unstable and that these strains turn the expression of flagella on and off.

The motility phenotypes of the Fla^+ and Fla^- strains received from D. Newell and R. Black were confirmed by streaking on semisolid medium (thioglycolate medium supplemented with 0.33% agar; BBL Microbiology Systems, Cockeysville, Md.). Rabbits were challenged with 5×10^8 to 5×10^9 CFU of each set of strains via the RITARD procedure (3). There were no significant differences in the characteristics or the time course of the infections observed. Of 18 animals challenged with strain 81116 Fla^-, 8 had mucous diarrhea compared with 5 of 11 animals challenged with 81116 Fla^+ which had mucous diarrhea. In rabbits challenged with A3249 Fla^+ or A3249 Fla^-, four of five animals in each group became ill.

Stools of infected animals were cultured on blood agar plates with campylobacter selective supplement (code SR98; Oxoid Ltd., Basingstoke, England). All cultures were incubated at 42°C in an atmosphere of 85% nitrogen, 10% CO_2, and 5% O_2. Motile campylobacter were seen in stool cultures from all animals challenged with aflagellated forms. Quantitative data were obtained from one group of six rabbits challenged with strain 81116 Fla^-. Individual campylobacter colonies were selected from 24-, 48-, 72-, and 96-h postinfection stool cultures and streaked on motility agar. At 24 h, 4 of 72 colonies isolated from the stool culture were motile; 58 of 72 colonies at 48 h and 70 of 72 colonies at 72 h were motile. Only motile colonies were recovered at 96 h.

The onset of diarrhea occurred 3 to 6 days postoperatively in all groups. The large variation in onset times seen in rabbits challenged with the same strain prevented us from determining if significant differences existed between groups. However, all rabbits were shedding only motile organisms by the end of the incubation period whether or not diarrhea occurred.

These data suggested that the Fla^- phenotype in strain 81116 was quite unstable. We used a pour-plate technique to determine the motility phenotype of large numbers of organisms from both strains (Fig. 1). Thioglycolate medium supplemented with 0.33% agar was autoclaved and cooled to 42°C. A suspension of bacteria was added to the medium to achieve a final density of approximately 500 colonies per plate. The plates were allowed to solidify at room temperature for 2 h and were incubated at 42°C for 48 h. Strains A3249 Fla^- and 81116 Fla^- were plated in motility pour-plate media, and single nonmotile colonies were selected and grown overnight on blood agar plates. The cells from the blood plate were suspended in saline, diluted, and plated in motility agar. Strains were passaged at least six times from single nonmotile colonies. As the final passage, sufficient cells were plated to examine 20,000 colonies per strain per experiment. The results are summarized in Table 1. Flagellated forms were observed in strains 81116 Fla^- and A3249 Fla^- at a rate of 4.0×10^{-7} and 8.0×10^{-7} per cell per generation, respectively. Strains A3249 Fla^+ and 81116 Fla^+ were similarly passaged and plated in semisolid motility medium. The transition from Fla^+ to Fla^- was much higher: 3.1×10^{-3} per cell per generation for strain 81116 and 5.9×10^{-3} per cell per generation for strain A3249. These rates should be considered approximate, because we observed some variability in duplicate experiments.

It is interesting to note that the frequency of the shift from Fla^+ to Fla^- is very high during growth in laboratory media. In contrast, the Fla^- to Fla^+ shift clearly predominated in the rabbit intestine. However, our data do not allow us to distinguish an increase in the in vivo Fla^- to Fla^+ rate from a positive selection for the flagellated form (e.g., enhanced intestinal adherence).

* Corresponding author.

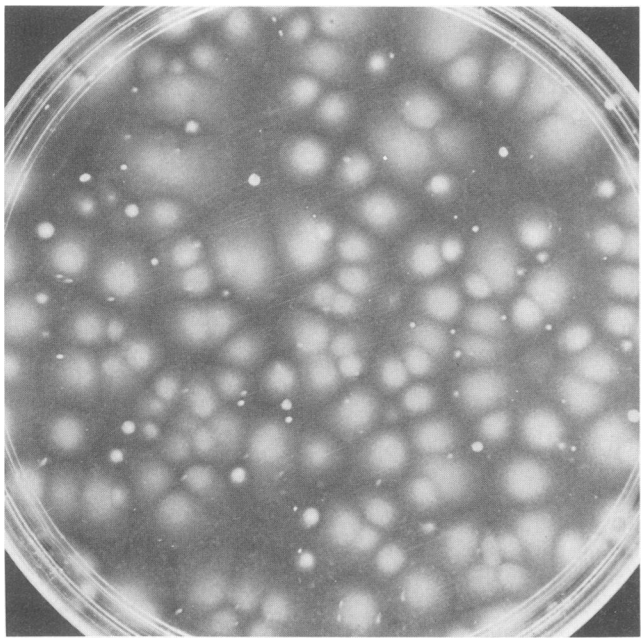

FIG. 1. Pour plate, strain A3249 Fla$^+$. The spreading colonies composed of flagellated organisms are readily distinguished from the compact colonies formed by aflagellated organisms. The phenotypes were confirmed by light microscopic observation of motility and stained flagella (8) and by transmission electron microscopy of negatively stained cells.

Flagella have been implicated as virulence determinants in other enteric pathogens. A role for flagella in the adherence of *Vibrio cholerae* to intestinal cells has been suggested (1, 6). Recently, flagella have been reported to enhance pathogenicity in *Salmonella typhimurium* (4, 23), probably by increasing survival time within macrophages. We can only speculate on the advantages gained by *C. jejuni* through the selective expression of flagella. The ability of bacteriophage Mu to vary its host range through a mechanism of genetic switching is well documented (22). Similarly, reversible gene expression is becoming increasingly recognized as a means by which pathogenic bacteria modulate important surface determinants. The classic example is the phase variation of flagellar antigen type in *S. typhimurium* (18, 20). Similar phase variations have been described for various virulence determinants in *Neisseria gonorrhoeae* (2, 11), *Bordetella pertussis* (15, 24), and uropathogenic *Escherichia coli* (5). The variation rates are, at least in some instances, affected by environmental conditions (14).

TABLE 1. Variation of motility phenotypes in *C. jejuni*

Strain	Variation	No. of variants	No. of total cells examined[a]	Rate[b]
81116	Fla$^-$→Fla$^+$	2	94,600	4.0×10^{-7}
	Fla$^+$→Fla$^-$	3,960	21,498	3.1×10^{-3}
A3249	Fla$^-$→Fla$^+$	3	59,600	8.0×10^{-7}
	Fla$^+$→Fla$^-$	9,100	25,640	5.9×10^{-3}

[a] The numbers for the Fla$^-$→Fla$^+$ transition are the totals from three separate experiments.

[b] A colony of a given phenotype harvested from motility agar was grown overnight on blood agar plates. Cells were suspended in normal saline, diluted, and plated in motility pour plates at a density sufficient to give approximately 500 colonies per plate. 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 (5, 18, 20).

In this study we looked at two strains of *C. jejuni*. The initial aflagellated variants were spontaneously occurring isolates. However, we demonstrated reversible expression of flagella in both directions from each isolate. These data suggest that the ability to reversibly express flagella is inherent in the wild-type strain and not just a peculiarity of the original Fla$^-$ variants. Whether most strains of *C. jejuni* are capable of this variation remains to be determined, but our preliminary observations indicate that flagellar phase variation occurs in other *C. jejuni* strains. It should be noted that Wenman et al. (25) have observed that strain 81116 Fla$^+$ is serotype 6 in the typing scheme of Lior et al. (9), while the Fla$^-$ variant is untypable in this scheme. Thus, a component of the flagella is a major antigenic component of serotype 6 specificity. The demonstration of reversible flagellar expression may have a significant impact on the interpretation of results obtained with the Lior serotyping scheme.

A direct role for flagella as virulence determinants of *C. jejuni* remains to be elucidated. Stable, congenic, flagellated and aflagellated mutants need to be compared in animal models. An analysis of gene expression at the molecular level should also increase our understanding of the involvement of flagella in the pathogenicity of *C. jejuni*. A more refined genetic understanding of flagellar antigen expression in *C. jejuni*, however, awaits the development of experimental genetic systems in this organism.

We gratefully acknowledge the many contributions of Robert Black and Dianne Newell. We also thank C. Blanco for technical assistance, Y. Kang for electron microscope preparations, and E. Weiss for critical comments on the manuscript.

This work was supported by the Naval Medical Research and Development Command, Research Work Units 3M162770A870AS309 and 3M463763A807. E.C.L. is a postdoctoral fellow of the National Research Council.

LITERATURE CITED

1. **Attridge, S. R., and D. Rowley.** 1983. The role of the flagellum in adherence of *Vibrio cholerae*. J. Infect. Dis. **147**:864–872.
2. **Buchanan, T. M.** 1975. Antigenic heterogeneity of gonococcal pili. J. Exp. Med. **141**:1470–1475.
3. **Caldwell, M. B., R. I. Walker, S. D. Stewart, and J. E. Rogers.** 1983. Simple adult rabbit model for *Campylobacter jejuni* enteritis. Infect. Immun. **42**:1176–1182.
4. **Carsiotis, M., D. L. Weinstein, H. Karch, I. A. Holder, and A. D. O'Brien.** 1984. Flagella of *Salmonella typhimurium* are a virulence factor in infected C57BL/6J mice. Infect. Immun. **46**:814–818.
5. **Eisenstein, B. I.** 1981. Phase variation of type I fimbriae in *Escherichia coli* is under transcriptional control. Science **214**:337–339.
6. **Guentzel, M. N., and L. J. Berry.** 1975. Motility as a virulence factor for *Vibrio cholerae*. Infect. Immun. **11**:890–897.
7. **Klipstein, F. A., and R. F. Engert.** 1984. Properties of crude *Campylobacter jejuni* heat-labile enterotoxin. Infect. Immun. **45**:314–319.
8. **Kodaka, H., A. Y. Armfield, G. L. Lombard, and V. R. Dowell, Jr.** 1982. Practical procedure for demonstrating bacterial flagella. J. Clin. Microbiol. **16**:948–952.
9. **Lior, H., D. L. Woodward, J. A. Edgar, L. J. Laroche, and P. Gill.** 1982. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. J. Clin. Microbiol. **15**:761–768.
10. **McCardell, B. A., J. M. Madden, and E. C. Lee.** 1984. *Campylobacter jejuni* and *Campylobacter coli*: production of a

cytotonic toxin immunologically similar to cholera toxin. J. Food Prot. **47**:943–949.
11. **Meyer, T. F., N. Mlawer, and M. So.** 1982. Pilus expression in *Neisseria gonorrhoeae* involves chromosomal rearrangement. Cell **30**:45–52.
12. **Newell, D. G., H. McBride, and A. D. Pearson.** 1984. The identification of outer membrane proteins and flagella of *Campylobacter jejuni*. J. Gen. Microbiol. **130**:1201–1208.
13. **Newell, D. G., and A. D. Pearson.** 1982. Pathogenicity of *Campylobacter jejuni*—an *in vitro* model of adhesion and invasion?, p. 196–199. *In* D. G. Newell (ed.), Campylobacter: epidemiology, pathogenesis and biochemistry. MTP Press Ltd., Lancaster, United Kingdom.
14. **Ottow, J. C. G.** 1975. Ecology, physiology and genetics of fimbriae and pili. Annu. Rev. Microbiol. **29**:79–108.
15. **Peppler, M. S.** 1982. Isolation and characterization of isogenic pairs of domed hemolytic and flat nonhemolytic colony types of *Bordetella pertussis*. Infect. Imun. **35**:840–851.
16. **Ruiz-Palacios, G. M., E. Escamilla, and N. Torres.** 1981. Experimental *Campylobacter* diarrhea in chickens. Infect. Immun. **34**:250–255.
17. **Ruiz-Palacios, G. M., J. Torres, N. I. Torres, E. Escamilla, B. R. Ruiz-Palacios, and J. Tamayo.** 1983. Cholera-like enterotoxin produced by *Campylobacter jejuni*: characterization and clinical significance. Lancet **ii**:250–253.
18. **Silverman, M., and M. Simon.** 1980. Phase variation: genetic analysis of switching mutants. Cell **19**:845–854.
19. **Spira, W. M., R. B. Sack, and J. L. Froehlich.** 1981. Simple adult rabbit model for *Vibrio cholerae* and enterotoxigenic *Escherichia coli* diarrhea. Infect. Immun. **32**:739–747.
20. **Stocker, B. A. D.** 1949. Measurements of rate of mutation of flagellar antigenic phase in *Salmonella typhimurium*. J. Hyg. **47**:398–413.
21. **Taylor, D. E., S. A. DeGrandis, M. A. Karmali, and P. C. Fleming.** 1981. Transmissible plasmids from *Campylobacter jejuni*. Antimicrob. Agents Chemother. **19**:831–835.
22. **Van de Putte, P., S. Cramer, and M. Giphart-Gassler.** 1980. Invertible DNA determines host specificity of bacteriophage Mu. Nature (London) **286**:218–222.
23. **Weinstein, D. L., M. Carsiotis, C. R. Lissner, and A. D. O'Brien.** 1984. Flagella help *Salmonella typhimurium* survive within murine macrophages. Infect. Immun. **46**:819–825.
24. **Weiss, A. A., and S. Falkow.** 1984. Genetic analysis of phase change in *Bordetella pertussis*. Infect. Immun. **43**:263–269.
25. **Wenman, W. M., J. Chai, T. J. Louie, C. Goudreau, H. Lior, D. G. Newell, A. D. Pearson, and D. E. Taylor.** 1985. Antigen analysis of *Campylobacter* flagellar protein and other proteins. J. Clin. Microbiol. **21**:108–112.