JOURNAL OF CLINICAL MICROBIOLOGY, July 1988, p. 1411–1413
0095-1137/88/071411-03$02.00/0

Vol. 26, No. 7

Identification of an Antigen Common to Different Species of the Genus *Campylobacter*

S. D. MILLS,† L. A. KURJANCZYK, AND J. L. PENNER*

Department of Microbiology, University of Toronto, Toronto, Ontario M5G 1L5, Canada

Received 24 February 1988/Accepted 13 April 1988

Affinity-purified antibody specific for a determinant on flagellin from *Campylobacter jejuni* was used to screen by immunoblotting strains of *C. coli*, *C. laridis*, *C. fetus*, "*C. upsaliensis*," *C. pylori*, and *C. sputorum* biovar fecalis. The antigen was detected in each of these species, but the molecular weights of the proteins bearing the common antigen varied considerably.

Investigations of the flagella of *Campylobacter jejuni* and *Campylobacter coli* in an earlier study (9) led to the discovery of an antigen that is common to all 42 serotype reference strains (serostrains) from the O-serotyping scheme of *C. jejuni* and to all 18 strains from the scheme for *C. coli* (12). The common antigen was identified through the use of a high-affinity antibody (HA-Ab) prepared against the *C. jejuni* serostrain for Pen 1. In immunoblotting experiments, the application of the HA-Ab to electrophoresed solubilized whole-cell preparations and acid-glycine extracts of each of the 60 serostrains led to the visualization of a band of approximately 62 kilodaltons (kDa) in each preparation and extract. Campylobacter proteins of this M_r have been identified as flagellin (5, 6, 11).

The common antigen, however, is apparently not surface exposed in the native state, as neither slide agglutinations of cell suspensions nor coagglutinations with protein A-bearing staphylococci can be demonstrated with the HA-Ab antibody (9). This suggested that the common antigen would not be useful as the basis for a vaccinogen but that it might be useful in rapid diagnostic procedures. In this report we describe further investigations to determine whether the common antigen is present only in *C. jejuni* and *C. coli* or whether it also occurs in strains belonging to other *Campylobacter* species.

An HA-Ab was prepared from a rabbit antiserum produced against extracted and purified flagellin of the Pen 1 serostrain as described previously (9). Briefly, antibody was obtained by ammonium sulfate precipitation of the rabbit antiserum, and immunoglobulin G was separated by ion-exchange chromatography (DEAE-Sephacel) at pH 7.0. The HA-Ab was isolated by passing the immunoglobulin G containing fraction through an affinity column in which flagellin had been coupled to CNBr-activated Sepharose. For use in immunoblotting, washed whole cells were solubilized in 1% sodium dodecyl sulfate, electrophoresed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (40 μg of protein per lane), and either electrophoretically transferred to nitrocellulose membrane or stained with Coomassie brilliant blue. Protein concentrations were determined by the method of Lowry et al. (7). The molecular size markers included lysosyme (14.4 kDa), soybean trypsin inhibitor (21. 5 kDa), carbonic anhydrase (31 kDa), ovalbumin (45 kDa), bovine serum albumin (66.2 kDa), and phosphorylase *b* (92.5 kDa). Nitrocellulose blots were blocked in 2.0% gelatin for 1 h and exposed to HA-Ab (2 μg/ml) in 2.0% gelatin overnight. The remainder of the procedure was in accordance with the immunoblotting kit provided by Bio-Rad Laboratories.

The results of examining catalase-positive *Campylobacter* isolates received in our laboratory classified as *C. laridis* (five strains), *C. fetus* subsp. *fetus* (five strains), *C. fetus* subsp. *venerealis* (six strains), *C. sputorum* biovar fecalis (formerly *C. fecalis*) (four strains), *C. pylori* (three strains), and "*C. upsaliensis*" (one strain) are shown in Table 1. For comparative and control purposes, three *C. jejuni* and three *C. coli* strains were also included. As shown in an earlier study (9), the HA-Ab recognized bands of 60 to 62 kDa in *C. jejuni* and *C. coli*. Similar results were obtained for the *C. laridis* strains. It should be noted that reactions with the HA-Ab were much stronger for the three thermophilic species than for strains of other species. For *C. sputorum* biovar fecalis, the HA-Ab recognized proteins also of 61 to 62 kDa but with much less intensity. Generally weaker reactions were observed for the *C. fetus* strains, and the proteins from three strains differed in having considerably lower molecular sizes of 52 to 53 kDa. The one strain of "*C. upsaliensis*" yielded a doublet; one band of 64 kDa that stained more intensely than a band of 60 kDa. Weakly staining doublets of 54 and 58 kDa were also seen for the

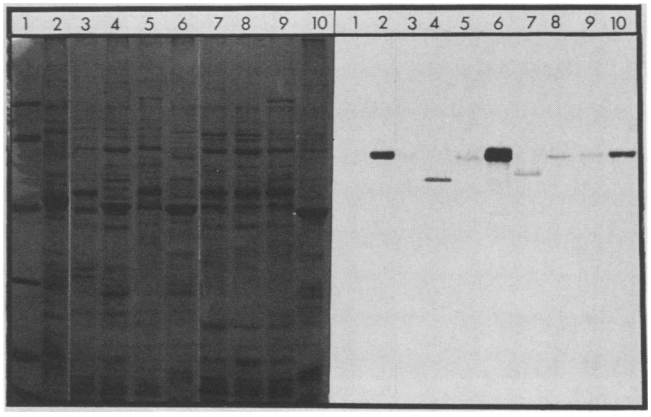

FIG. 1. Coomassie brilliant blue-stained electrophoresed proteins (left) and results of immunoblotting (right) of whole-cell preparations from various strains of *Campylobacter* spp. Lanes: 1, molecular weight markers; 2, *C. coli* PC354; 3 to 5, *C. fetus* subsp. *venerealis* PC89, PC19, and PC2; 6, *C. jejuni* PC23; 7, 8, and 9, *C. fetus* subsp. *fetus* PC11, PC9, and PC3; 10, *C. jejuni* MK5.

* Corresponding author.
† Present address: Département de Biochimie Médicale, Centre Médicale Universitaire, Geneva, Switzerland.

TABLE 1. Results of immunoblotting electrophoresed proteins from strains of different *Campylobacter* species

Species	Strain(s)	Mol size (kDa)	Intensity[a] of reaction
C. jejuni	MK5 (Pen 1), PC23	62	+++
	MK7 (Pen 4)	60	+++
C. coli	PC66 (Pen 24)	61	+++
	PC70 (Pen 25), PC354 (Pen 54)	62	+++
C. laridis	85-18026, PC531, PC637, PC658	61	+++
	PC557	62	+++
C. fetus subsp. *venerealis*	18799, 14840, PC89	62	+
	PC2	62	++
	18652	53	+
	PC19	52	++
C. fetus subsp. *fetus*	PC15	62	++
	PC3, PC9, PC14	62	+
	PC11	53	+
"*C. upsaliensis*"	MIC17399	64, 60[b]	++, +
C. sputorum biovar fecalis	EF12014, CCUG 17695B	62	+
	EF12015	61	+
	CCUG 17761	61	±
C. pylori	CP558, CP656, CP571	58, 54[b]	±, +

[a] Intensity of reactions in immunoblots: +++, strong (intense dark-purple bands); ++, moderate (purple bands easily observed); +, weak (light purple bands); ±, faint band sometimes observed.
[b] Doublet.

three *C. pylori* strains. The presence of doublets in a *C. coli* strain has been attributed to its ability to synthesize two antigenic types of flagellin referred to as phase 1 (61.5 kDa) and phase 2 (59.5 kDa) (4).

C. pylori, unlike other *Campylobacter* species, has tufts of four to six sheathed flagella (2, 3). The observation that each of the three strains in this study displayed the phenomenon of doublets also suggested that two types of flagella are capable of being synthesized by this species. However, antisera were not prepared against *C. pylori*, and at this point it cannot be concluded that the doublets represent flagella of different antigenicity. Nor is it known that the three strains belong to different serotypes. Representative results for all the species are shown in Fig. 1 and 2.

From these results it is evident that the common antigen is not a distinctive feature of *C. jejuni* and *C. coli* but is also well expressed in *C. laridis* and less well expressed in other species. Nor is the common antigen likely to be restricted to the genus *Campylobacter*, as it occurs in *C. pylori*, for which evidence continues to accumulate indicating that it belongs to another genus (14). The antigen detected in these experiments therefore probably reflects a conserved epitope in the flagella of *Campylobacter* species and other genera that have flagella of similar structure.

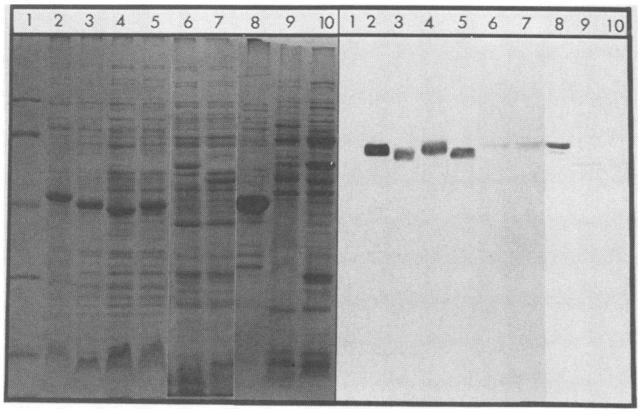

FIG. 2. Coomassie brilliant blue-stained electrophoresed proteins (left) and results of immunoblotting (right) of whole-cell preparations from various strains of *Campylobacter* spp. Lanes: 1, molecular weight markers; 2, *C. coli* PC354; 3 to 5, *C. laridis* 85-18026, PC557, and PC531; 6 and 7, *C. sputorum* biovar fecalis EF12014, CCUG 17695B; 8, "*C. upsaliensis*" MIC17399; 9 and 10, *C. pylori* CP656 and CP558.

This is not surprising in light of the new information on the amino acid sequences of *C. jejuni* and *C. coli* flagellin that has recently become available through the studies of Logan et al. (6). Evidently there is sequence homology not only between the N-terminal ends of the flagellin molecules of *C. jejuni* and *C. coli* but also with flagellin from *Bacillus subtilis* and *Salmonella typhimurium*, species unrelated to *Campylobacter* species.

The common antigen was shown to stimulate the production of antibodies during the course of an infection with *C. jejuni* (8, 10). Acid-glycine extracts of *C. jejuni* that are currently in use as the test antigens in serodiagnosis (1, 13, 15) would be expected to contain this antigen along with other common antigens and serotype-specific proteins. The success of the acid-glycine extract in detecting serum antibodies is therefore at least partly due to the presence of the common antigen associated with flagellin. Since our data suggest that the antibodies detected in human sera could result from exposure to strains belonging to species other than *C. jejuni* and *C. coli*, interpretations of serodiagnostic tests that use the acid-glycine extracts should take into account the possibility that antibody levels detected in patient sera may not always exclusively reflect infections with these two species.

We are grateful to B. D. Firehammer, P. C. Fleming, and M. A. Karmali for contributing strains and to T. Carroll for typing the manuscript.

The study was supported by a grant from the Medical Research Council of Canada. S.D.M. is the recipient of an Ontario Ministry of Health postdoctoral fellowship.

LITERATURE CITED

1. **Blaser, M. J., and D. J. Duncan.** 1984. Human serum antibody response to *Campylobacter jejuni* infection as measured in an

enzyme-linked immunosorbent assay. Infect. Immun. **44**:292–298.
2. **Goodwin, C. S., R. K. McCulloch, J. A. Armstrong, and S. H. Wee.** 1985. Unusual cellular fatty acids and distinctive ultrastructure in a new spiral bacterium (*Campylobacter pyloridis*) from the human gastric mucosa. J. Med. Microbiol. **19**:257–267.
3. **Jones, D. M., A. Curry, and A. J. Fox.** 1985. An ultrastructural study of the gastric Campylobacter-like organism '*Campylobacter pyloridis*.' J. Gen. Microbiol. **131**:2335–2341.
4. **Harris, L. A., S. M. Logan, P. Guerry, and T. J. Trust.** 1987. Antigenic variation of *Campylobacter* flagella. J. Bacteriol. **169**: 5066–5071.
5. **Lee, A., S. M. Logan, and T. J. Trust.** 1987. Demonstration of a flagellar antigen shared by a diverse group of spiral-shaped bacteria that colonize intestinal mucus. Infect. Immun. **55**:828–831.
6. **Logan, S. M., L. A. Harris, and T. J. Trust.** 1987. Isolation and characterization of *Campylobacter* flagellins. J. Bacteriol. **169**: 5072–5077.
7. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
8. **Mills, S. D., and W. C. Bradbury.** 1984. Human antibody response to outer membrane proteins of *Campylobacter jejuni* during infection. Infect. Immun. **43**:739–743.
9. **Mills, S. D., W. C. Bradbury, and J. L. Penner.** 1986. Isolation and characterization of a common antigen in *Campylobacter jejuni* and *Campylobacter coli*. J. Clin. Microbiol. **24**:69–75.
10. **Nachamkin, I., and A. M. Hart.** 1985. Western blot analysis of the human antibody response to *Campylobacter jejuni* cellular antigens during gastrointestinal infection. J. Clin. Microbiol. **21**:33–38.
11. **Nachamkin, I., and A. M. Hart.** 1986. Common and specific epitopes of *Campylobacter* flagellin recognized by monoclonal antibodies. Infect. Immun. **53**:438–440.
12. **Penner, J. L., J. N. Hennessy, and R. V. Congi.** 1983. Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of thermostable antigens. Eur. J. Clin. Microbiol. **2**:378–383.
13. **Rautelin, H. I., and T. U. Kosunen.** 1987. Campylobacter etiology in human gastroenteritis demonstrated by antibodies to acid extract antigen. J. Clin. Microbiol. **25**:1944–1951.
14. **Romaniuk, P. J., B. Zoltowska, T. J. Trust, D. J. Lane, G. J. Olsen, N. R. Pace, and D. A. Stahl.** 1987. *Campylobacter pylori*, the spiral bacterium associated with human gastritis, is not a true *Campylobacter* sp. J. Bacteriol. **169**:2137–2141.
15. **Svedhem, A., H. Gunnarsson, and B. Kaijser.** 1983. Diffusion-in-gel enzyme-linked immunosorbent assay for routine detection of IgG and IgM antibodies to *Campylobacter jejuni*. J. Infect. Dis. **148**:82–92.