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Common and Specific Epitopes of *Campylobacter* Flagellin Recognized by Monoclonal Antibodies

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Murine monoclonal antibodies to *Campylobacter jejuni* recognized a flagellin epitope common to most *Campylobacter* species and an epitope restricted to *C. jejuni* and *C. coli*. These epitopes are distinct from the serotype-specific epitope recently detected on the flagellin and have not been described previously.

The flagellin protein of *Campylobacter jejuni* appears to be one of the immunodominant proteins expressed during gastrointestinal infection (9, 13, 20). A study by Newell (15) shows that *C. jejuni* may attach to eucaryotic cells via an as yet undefined adhesin that could be associated with the flagellum. A more recent study by Morooka et al. (10) shows that the motility of *C. jejuni* is an important factor in the intestinal colonization of suckling mice. Other studies on *Vibrio cholerae* (1) *Salmonella typhimurium* flagella (3, 19) suggest that the flagellum may be important as a virulence factor in the pathogenesis of these infections. Further studies on experimental cholera vaccines containing flagellar components suggest that flagellin may elicit protective antibodies against *V. cholerae* (17, 21) and thus provide additional evidence for a role of the flagellum in infection and immunity.

Studies on *C. jejuni* and *C. coli* have focused on surface components that may be important to the virulence of these organisms. *C. jejuni* exhibits antigenic variability in cellular antigens as evidenced by the existence of multiple serotype antigens (8, 16). Numerous serotypes are distributed in human disease, as well as in natural reservoirs (4, 11). The flagellin protein has been recently described as possessing the serotype-specific epitope of *C. jejuni* and *C. coli* (W. N. Wenman, D. E. Taylor, and H. Lior, 3rd International Workshop on *Campylobacter* Infections, Ottawa, Canada, abstr. no. 128, 1985) responsible for the serotyping scheme of Lior et al. (8).

In developing immunologic tools for studying the primary structure of campylobacter flagellin, we were able to define two distinct epitopes on campylobacter flagellin that define a common genus-specific determinant and a determinant restricted to *C. jejuni* and *C. coli*. This is in addition to the already known serotype specificity of the flagellin protein.

Murine monoclonal antibodies were produced using a standard fusion protocol described by Kennett (6) by immunizing mice with whole formalinized *C. jejuni* VZ. Immune spleen cells were harvested and fused with an Sp2/0 plasmacytoma cell line by using polyethylene glycol. Culture supernatants were screened for binding to *C. jejuni* by solid-phase immunoassay essentially as described previously (12) except that peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG) was used rather than ^{125}I-labeled protein A.

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Antibodies from clones 10-44 and 10-37 initially showed binding to a determinant(s) on most *C. jejuni* strains and were chosen for further studies. Both antibodies were of the IgG1 isotype as determined by immunoelectrophoresis by using subclass-specific antisera (Litton Bionetics, Kensington, Md.). A BioDot assay was used to assess the reactivity of monoclonal antibodies to a variety of bacterial strains. Briefly, nitrocellulose membranes (BA85; Schleicher & Schuell, Inc., Keene, N.H.) were loaded with whole suspensions of bacteria using a BioDot apparatus (Bio-Rad Laboratories, Richmond, Calif.) according to manufacturer instructions. Membranes were blocked in 3% bovine serum albumin-10 mM Tris-0.9% NaCl buffer for 2 h at room temperature. Hybridoma culture supernatants (undiluted) were added and incubated for 1.5 at room temperature. Membranes were washed four times with 1% bovine serum albumin-Tris-NaCl buffer. Antibody binding was detected by adding 5 μCi of ^{125}I-labeled goat anti-mouse IgG and incubated for 1.5 h at room temperature. The membranes were washed, dried, and exposed to X-ray film for 24 to 72 h at −70°C, and autoradiograms were developed. A positive reaction showed a dark spot on the film, and a negative reaction showed no darkening of the film. Positive and negative controls were run with each assay and did not indicate any nonspecific binding.

Table 1 shows the reactivity patterns of the two hybridoma clones with *Campylobacter* isolates. Clone 10-37 reacted with 79 (98%) of 81 *C. jejuni* isolates tested. These isolates were obtained from humans with diarrheal disease, and two others were isolated from hamsters with proliferative ileitis. At least 12 isolates were known to differ in flagellum serotype specificity (13); thus, an epitope distinct from the serotype epitope was detected. Antibodies from these clones also reacted with all strains of *C. coli*. Antibody from clone 10-44, in contrast, reacted with 80 (99%) of the *C. jejuni* isolates and other campylobacter species. Antibodies from both clones did not react with 91 isolates of bacteria from a broad range of genetically diverse bacteria. However, monoclonal antibody from clone 10-37 did react with one strain of *S. aureus* and was thought to represent nonspecific binding by protein A rather than specific immunologic cross-reactivity.

To determine the specific epitope reactive with these antibodies, we performed Western blotting with whole *C. jejuni* and purified flagellin protein as described previously (13) except that ^{125}I-labeled goat anti-mouse IgG was used rather than protein A (Fig. 1). Antibody from clone 10-37 (panel A) reacted strongly with flagellin in whole cells of *C.*

TABLE 1. Reactivity of monoclonal antibodies against *Campylobacter* spp.

Organism	No. of strains tested	No. of strains reactive with Monoclonal antibody	
		10-44	10-37
C. jejuni (human)	79	78	77
C. jejuni (hamster)	2	2	2
C. coli[a,b]	12	12	12
C. fetus subsp. *fetus*[b]	2	2	0
C. fetus subsp. *venerealis*[b]	2	2	0
C. laridis[b,c]	3	3	0
C. sputorum ("faecalis")[b]	2	2	0
Enterobacteriaceae strains	64	0	0
Pseudomonas sp.	10	0	0
Vibrio and *Aeromonas* spp.	6	0	0
Staphylococci, streptococci	11	0	1

[a] 10 strains obtained from C. Patton, Centers for Disease Control, Atlanta, Ga.

[b] Isolates obtained from R. M. Smibert, Virginia Polytechnic Institute and State University, Blacksburg, Va.

[c] One strain of *C. laridis* isolated from human source (14).

jejuni but also showed slight reactivity with a lower-molecular-weight component. Similarly, antibodies from clone 10-44 (panel B) showed predominant reactivity with flagellin and showed some reactivity with a lower-molecular-weight component present in the whole-cell preparation. Although the identity of this component has not been firmly established, we believe this represents a fragment from degraded flagellin based on freeze-thaw experiments performed in the laboratory (data not shown). It is unlikely that this was a reaction with some other component, such as a major outer membrane protein, due to the presence of a mixture of antibodies because the hybridomas had been recloned to ensure monoclonality during the initial production procedure.

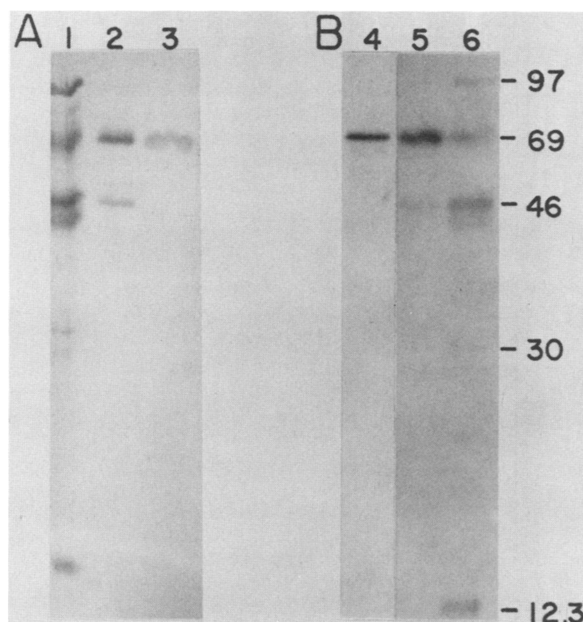

FIG. 1. Western blot analysis of monoclonal antibodies against whole *C. jejuni* and purified flagellin from human strain VZ. (A) Analysis of antibodies from clone 10-37. Lanes: 1, ^{14}C-labeled molecular-weight standards (New England Nuclear Corp., Boston, Mass.); 2 and 3, whole solubilized *C. jejuni* and purified flagellin, respectively. (B) Analysis of antibodies from clone 10-44. Lanes: 4 and 5, purified flagellin and whole solubilized *C. jejuni*, respectively; 6, ^{14}C-labeled molecular-weight standards.

Monoclonal antibodies against *C. jejuni* have been reported by Kosunen et al. (7) and showed reactivity patterns that suggested the presence of common determinants present in undefined extracts of whole bacteria. However, common epitopes of campylobacter flagellin have not been described previously.

The cross-reactivity observed with these antibodies with *C. jejuni* and *C. coli* are not surprising since both have a high degree of interspecies relatedness and are the most genetically related species within the genus *Campylobacter* (2, 18). Clinically, *C. coli* causes diarrheal disease indistinguishable from *C. jejuni* (5) and accounts for 3 to 5% of the isolates from human gastroenteritis. Other species tested in this study are also known to cause human infections. *C. fetus* subsp. *fetus* is known to cause septicemia (5) and *C. laridis* has been implicated as a rare cause of human infection (14).

The role of flagellin in the pathogenesis of campylobacter infection is not known at this time. The results of this study show that campylobacter flagellin possesses multiple epitopes that define different specificities within the genus. Because campylobacter flagellin appears to be immunodominant in patients with infection, the role of antibodies against common epitopes of campylobacter should be studied in relation to protection against infection. Such studies are ongoing in this laboratory.

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