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Monoclonal Antibodies Specific for Hippurate Hydrolase of *Campylobacter jejuni*

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Eleven monoclonal antibodies raised against recombinant *Campylobacter jejuni* hippurate hydrolase were tested for binding to lysates from 19 *C. jejuni* strains, 12 other *Campylobacter* strains, and 21 non-*Campylobacter* strains. Several monoclonal antibodies bound to *C. jejuni* but not to other *Campylobacter* species and may be useful in a species-specific immunoassay.

Campylobacter spp. are commonly implicated in gastroenteritis (1). *Campylobacter jejuni* is the species of *Campylobacter* most frequently associated with disease in humans, but other species are also capable of causing illness (6). Identification of *Campylobacter* isolates to the species level is important, both for distinguishing pathogenic species from those not associated with disease in humans and for studying the epidemiology of *Campylobacter*-induced illness. There is a need for a rapid, easy-to-use test, such as an immunoassay, for identification of suspect *Campylobacter* isolates to the species level. The ability of *C. jejuni* to hydrolyze *N*-benzoylglycine (hippurate) to benzoic acid and glycine is commonly used to distinguish it from other *Campylobacter* species (7). This ability is due to the presence of a hippurate hydrolase enzyme (EC 3.5.1.32) which appears to be unique to *C. jejuni* (3), suggesting that it would make a good target for an immunoassay used to identify *C. jejuni.* The purpose of this study was to generate monoclonal antibodies specific for the hippurate hydrolase of *C. jejuni*. The specificity of the monoclonal antibodies was determined by screening using enzyme-linked immunosorbent assays (ELISAs) and Western blots of whole-cell lysates from strains of *C. jejuni*, other *Campylobacter* species, other enteric bacteria, and other hippurate hydrolase-positive non-*Campylobacter* bacteria. The same strains were tested for hippurate hydrolase activity (4) and were screened by colony blotting and Southern blotting for binding to a DNA probe shown to be specific for the hippurate hydrolase enzyme of *C. jejuni* (3).

Glutathione *S*-transferase (GST)-tagged recombinant hippurate hydrolase was produced by amplifying the *hipO* gene of *C. jejuni* ATCC 43431 by PCR (with the primers 5′CTCGGATCC ATGAATTTAATTCCAGAA3′ and 5′GAGGAATTCTTATT TTAAGTATTTTAAAG3′), using pHipO (3) as a template and introducing *Bam*HI and *Eco*RI sites on either side of the gene. The amplified DNA was subcloned into *Bam*HI and *Eco*RI sites of pGEX-2T (Amersham Pharmacia Biotech Inc., Baie d'Urfé, Quebec, Canada) and introduced into *Escherichia coli* JM101 by $CaCl_2$ transformation (Bulk Redi-Pack GST Purification Modules; Amersham Pharmacia). The fusion protein was produced in *E. coli* and purified by glutathione-Sepharose affinity chromatography (Bulk Redi-Pack GST Purification Modules; Amersham Pharmacia). Fifty-microgram aliquots of GST-fusion protein in Freund's incomplete adjuvant were used to immunize adult BALB/c mice subcutaneously three to five times, at 2-week intervals. Monoclonal antibodies were prepared using polyethylene glycol fusion and hypoxanthine-aminopterin-thymidine supplement to select for fused cells (2, 5). Culture supernatants were screened for antibody binding to GST-hippurate hydrolase by using 3 μg of the antigen ml^{-1} on ELISAs and 0.75 μg of the antigen per lane on Western blots. Cells from positive wells were cloned twice by limiting dilution. Eleven monoclonal antibodies that recognized GST-hippurate hydrolase were isolated, and culture supernatants from these antibodies were harvested and frozen at −20°C. All of the antibodies were determined to be immunoglobulin G1 isotype using the Isostrip Mouse Monoclonal Antibody Isotyping Kit (Roche Diagnostics; Laval, Quebec, Canada).

Whole-cell lysates used to screen the monoclonal antibodies by ELISA and Western blotting were prepared from 19 strains of *C. jejuni* (ATCC 43431, ATCC 49349, ATCC 29428, and 16 nonreference strains), six nonreference strains of *C. coli*, six strains of *C. lari* (ATCC 35221, NCTC 11352, and four nonreference strains), five strains of *C. hyointestinalis* (ATCC 35212, ATCC 25217, and three nonreference strains), six strains of *C. upsaliensis* (ATCC 43954 and five nonreference strains), one strain of *C. mucosalis* (ATCC 43264), one strain of *Arcobacter butzleri* (ATCC 49616), two strains of *E. coli* (ATCC 25922 and ATCC 43894), two strains of *Salmonella* spp. (ATCC13076 and ATCC 8391), two strains of *Shigella* spp. (ATCC 12022 and ATCC 25931), five strains of *Listeria monocytogenes* (ATCC 19115 and four nonreference strains), five strains of *Listeria innocua* (ATCC 33091 and four nonreference strains), one nonreference strain of *Corynebacterium matruchotii*, two strains of *Streptococcus agalactiae* (ATCC 12386 and ATCC 13813), and one nonreference strain of *Streptococcus uberis*. Nonreference strains were isolated from food, environmental, or clinical samples and identified by established biochemical methods. Strains of *C. jejuni*, *L. monocytogenes*, *L. innocua*, *C. matruchotii*, *S. agalactiae*, and *S. uberis*, but not the other species, were found to possess hippurate hydrolase activity. To prepare whole bacterial lysates, the bac-

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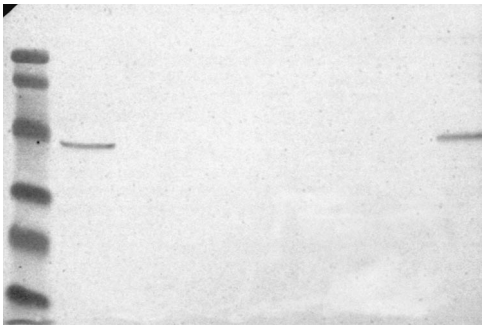

FIG. 1. Monoclonal antibody 364-10-9 binding of whole-cell lysates of *C. jejuni*, other *Campylobacter* species, and other bacterial species. Lane 1, prestained low-molecular-weight standards; lane 2, *C. jejuni* ATCC 43431; lane 3, *C. jejuni* ATCC 43431 hip$^-$ mutant; lane 4, *C. coli*; lane 5, *C. lari* ATCC 35221; lane 6, *C. hyointestinalis*; lane 7, *E. coli* ATCC 25922; lane 8, *L. monocytogenes* ATCC 19119.

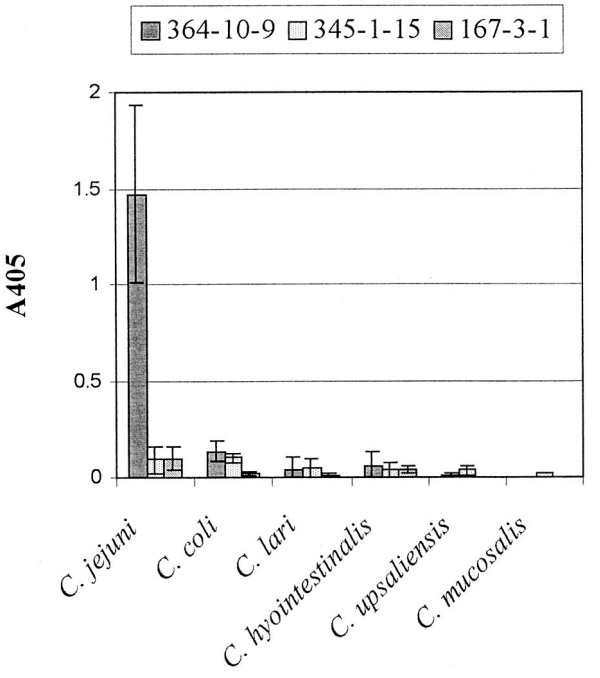

FIG. 2. Binding of monoclonal antibodies 364-10-9, 345-1-15, and 167-3-1 to whole-cell lysates of *Campylobacter* species by ELISA. Columns represent the average ± standard deviation of the average values obtained from all strains of each species tested. Each average value was originally determined from four replicate tests after subtraction of sample blank values. The number of strains tested for *C. jejuni*, *C. coli*, *C. lari*, *C. hyointestinalis*, *C. upsaliensis*, and *C. mucosalis* was 19, 6, 6, 5, 6, and 1, respectively.

teria were grown on Trypticase soy agar plus 5% sheep blood agar at 37°C for 24 h. *Campylobacter* strains were grown in anaerobic jars that were evacuated and filled three times with a microaerophilic gas mixture. Bacteria were harvested, suspended in phosphate-buffered saline, lysed by sonication, and cleared by centrifugation at 12,000 × *g* for 5 min in a microcentrifuge. The lysates were used as antigens on ELISAs and Western blots at 100 μg ml^{-1} and 40 μg per lane, respectively.

The monoclonal antibodies could be arranged into three groups, based on their species specificities. Group I contained three monoclonal antibodies that bound to lysates from *C. jejuni* strains and from two out of five strains of the hippurate hydrolase-positive *L. monocytogenes* strains tested. Group II contained five monoclonal antibodies that bound not only to lysates from *C. jejuni* strains and from the two *L. monocytogenes* strains but also bound to lysates from other *Campylobacter* species and the closely related *A. butzleri*. Group III contained three antibodies. All three antibodies showed a poor ability to distinguish *C. jejuni* from other species on ELISAs. On Western blotting, however, one of the antibodies from group III recognized lysates only from *C. jejuni* strains and the two *L. monocytogenes* strains, and the remaining two antibodies, 345-1-15 and 167-3-1, recognized lysates only from *C. jejuni* and not from the two *L. monocytogenes* strains. The antibodies recognized a single protein of 42.4 ± 0.8 kDa (average ± standard deviation) on Western blots of *C. jejuni* lysates. This was similar to the predicted molecular mass of the hippurate hydrolase enzyme (3). In the two *L. monocytogenes* strains, a single protein of 46.7 ± 0.1 kDa was recognized by the antibodies. The proteins recognized in the other *Campylobacter* strains and the *A. butzleri* strain were of variable sizes. None of the antibodies recognized whole-cell lysates from *C. jejuni* strain ATCC 43431 in which the *hipO* gene was interrupted by insertion of a kanamycin resistance gene. A summary of the binding of the group I monoclonal antibody 364-10-9 to Western blots of the strains tested in this study is shown in Fig. 1.

Colony blots and Southern blots were prepared as described elsewhere (DIG User's Guide for Filter Hybridization; Boehringer Mannheim GmbH), hybridized overnight at 42°C in standard buffer plus 50% formamide containing 37.5 ng of probe ml^{-1} and using UV light to cross-link DNA to nylon membranes. Southern blots were prepared from 5 μg of *Hin*dIII-digested genomic DNA per lane as described previously (8) and quantitated with the PicoGreen quantification reagent for double-stranded DNA (Molecular Probes, Eugene, Oreg.). The *hipO*-specific DNA probe was prepared by digesting a pUC19 plasmid containing a 0.8-kb *Hin*dIII fragment of *hipO* (3) with *Hin*dIII, gel purifying the fragment, and labeling it with digoxigenin using a Digoxigenin DNA Labeling and Detection Kit (Boehringer Mannheim, Indianapolis, Ind.). Blots were developed using a DIG Luminescent Detection Kit for Nucleic Acids (Boehringer Mannheim) with CSPD as substrate. The colony blots showed strong binding of the probe only to *C. jejuni* strains. A single band of 1.71 to 2.43 kb was observed on Southern blots of *Hin*dIII-digested genomic DNA isolated from *C. jejuni* strains.

Several of the monoclonal antibodies showed promise for use in an immunoassay. The group I monoclonal antibody 364-10-9 generated a strong signal on both ELISAs and Western blots of *C. jejuni* whole-cell lysates but did not display detectable binding to lysates from other *Campylobacter* species, suggesting that this antibody had the potential to be used in an assay for identification of *C. jejuni* among presumptive *Campylobacter* spp. isolates. While this antibody also recognized two of the hippurate hydrolase-positive *L. monocytogenes* strains tested, this binding would not interfere with an assay used for identification of *C. jejuni* from pure cultures of potential *Campylobacter* spp. isolates, since *Listeria* spp. are

unlikely to be coisolated on selective media with *Campylobacter* spp. The binding of 364-10-9 to the *L. monocytogenes* strains did not correlate with hippurate hydrolase activity, as all of the *Listeria* spp. isolates included in the study were hippurate hydrolase-positive and only two strains were recognized by the monoclonal antibodies. The protein recognized by 364-10-9 in these strains could be a different enzyme isotype or an unrelated protein with structural similarity to hippurate hydrolase. The colony blotting and Southern blotting confirmed that the *hipO* gene appears to be restricted to *C. jejuni* and is absent from other hippurate hydrolase-positive bacteria, such as the two *L. monocytogenes* strains.

The two group III monoclonal antibodies that recognized the *C. jejuni* strains but did not recognize the two *L. monocytogenes* strains on Western blotting, 345-1-15 and 167-3-1, might also be used in an assay for identification of *C. jejuni* isolates. The usefulness of these two monoclonal antibodies, however, is limited by the failure of these antibodies to differentiate *C. jejuni* from other *Campylobacter* species by ELISA (Fig. 2).

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