Identification of *Escherichia coli* Serotype O157 Strains by Using a Monoclonal Antibody†

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The O157 antigenic determinant of *Escherichia coli* serotype O157:H7, an important bacterial pathogen, resides in the polysaccharide portion of its cellular lipopolysaccharide component which, from structural studies, was identified as a linear polymer of repeating tetrasaccharide unit composed of β-glucose, 1-fucose, 2-acetamido-2-deoxy-D-galactose, and 4-acetamido-4,6-dideoxy-D-mannose residues (1:1:1:1). Hybrid cells producing monoclonal antibodies against the *E. coli* O157 antigen were obtained by fusion of myeloma cells with lymphocytes from BALB/c mice immunized with killed *E. coli* O157:H7 cells. Clones were selected for binding specificity with purified O polysaccharide. One monoclonal antibody used in direct slide agglutinations or in coagglutination reactions with *Staphylococcus aureus* Cowan 1 cells sensitized with the affinity column-purified antibody accurately detected all strains of *E. coli* O157 tested. This selected monoclonal antibody did not agglutinate *E. coli* strains such as *E. coli* O7 and *E. coli* O116 or other bacteria which are known to give positive agglutinations with conventional polyclonal *E. coli* antisera. These results indicate that the monoclonal antibody is a superior specific-typing reagent.

Several serotypes of *Escherichia coli* which produce verotoxins or Shiga-type cytotoxins have been isolated from cases of hemorrhagic colitis and associated with hemolytic-uremic syndrome (19, 20), and among these, *E. coli* serotype O157:H7 was first reported in 1982 in two outbreaks of hemorrhagic colitis in the United States (31, 37) and in one outbreak in Canada (18). Interest in the organism increased when it was found in sporadic cases and in outbreaks of hemorrhagic colitis in large numbers of hemolytic-uremic syndrome cases (11, 16, 17, 19, 26, 29, 30, 33, 34, 36). In a recent survey of stool specimens submitted for enteric bacteriological examination, *E. coli* O157:H7 was the third most frequently isolated pathogen, following *Campylobacter jejuni* and *Salmonella* spp., and was consistently isolated more frequently than *Shigella* spp. or *Yersinia enterocolitica* (7). Raw hamburger (37) has been implicated as a source of *E. coli* O157:H7, and the organism has been identified in the feces of cattle (1, 23).

In view of the evidence linking *E. coli* serotype O157:H7 with cases of hemorrhagic colitis, it is important to develop systems for the isolation and identification of the organism in foods and feces, particularly in the presence of other strains of *E. coli*. Laboratory screening methods which are based on the lack of sorbitol fermentation by these organisms at 24 h (15, 24) and on agglutinations with antisera against the H7 and O157 antigens (15) have been used. However, use of polyclonal rabbit *E. coli* O157 antisera can result in false identification (23), and serological cross-reactions (35) may have serious implications in the serodiagnostic identification of *E. coli* O157:H7. The structural basis of these cross-reactions resides in an O polysaccharide epitope that is found in several gram-negative lipopolysaccharides (LPS), and the detailed chemical structures of many of these antigens are now known.

In the case of the serotype O157 strains of *E. coli*, the O polysaccharide is an unbranched linear polymer of repeating tetrasaccharide units composed of β-glucose, 1-fucose, 2-acetamido-2-deoxy-D-galactose, and 4-acetamido-4,6-dideoxy-D-mannose residues (13, 28). Observed serological cross-reactions between *E. coli* serotype O157:H7 and other bacterial species (23) such as *Brucella abortus* (10), *Brucella melitensis* (3, 4), *Y. enterocolitica* serotype O:9 (9), *Salmonella* group N (O:30) (6, 27), and *Pseudomonas maltophilia* 555 (12) can be related to a common epitope involving N-acyl derivatives of 4-amino-4,6-dideoxy-a-D-mannopyranosyl residues in the O polysaccharide components of their respective smooth LPS (5).

The structural identification of the *E. coli* serotype O157 O polysaccharide and those of serological cross-reacting LPS has laid the foundation for the production of an *E. coli* O157 antigen-specific monoclonal antibody, since significant epitopic differences between the antigens were evident. This paper records the production and analysis of monoclonal antibodies which specifically react with *E. coli* serotype O157 isolates.

MATERIALS AND METHODS

Antigens. Purified *E. coli* serotype O157:H7 LPS and its O polysaccharide moiety were made as previously described (28).

Immunization. Mice were immunized with cells of *E. coli* O157:H7 (LCDC 82-1933; NRCC 4125) grown to late log phase in brain heart infusion medium (Difco Laboratories, Detroit, Mich.) in baffled 250-ml Erlenmeyer flasks shaken at 200 rpm at 37°C. Phenol (1%, wt/vol)-killed cells were washed with 0.01 M phosphate-buffered saline (PBS) (pH 7.0) and were suspended in PBS.

Female 6- to 8-week-old BALB/c mice (Charles River Canada Inc., St. Constant, Quebec, Canada) were given two intraperitoneal injections of 0.5 ml of suspended *E. coli* serotype O157:H7 cells, with 1 week between injections; after a 3-week rest, the mice received two final intravenous

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injections of 0.2 ml of the cell suspension, with 10 days between injections. All injections consisted of 10^8 phenol-killed cells in PBS.

**Fusions, cloning, and ELISA screening.** Spleen cells from two immunized mice were fused with a non-immunoglobulin-producing Sp2/O plasmacytoma cell line (32) (Institute for Medical Research, Camden, N.J.) in a ratio of 10:1 (8). Putative hybrids were screened by enzyme-linked immunosorbent assay (ELISA) on supernatants 10 to 14 days post-fusion.

Linbro enzyme immunoassay microtitration plates (Flow Laboratories, Mississauga, Ontario, Canada) were coated with *E. coli* serotype O157:H7 LPS solution (10 μg/ml in 0.05 M sodium carbonate, pH 9.8) by incubation at 37°C for 3 h. Bound antibody was detected by alkaline phosphatase-conjugated protein A (Dimension Laboratories, Inc., Mississauga, Ontario, Canada) used in conjunction with p-nitrophenyl phosphate, and the A_405 was read after 60 min of incubation at 20°C by using a Titer-tek Multiscan (Flow Laboratories). Hybrids with culture supernatants giving absorbance readings greater than 0.2 against a negligible background were cloned in semisolid agar by using mouse spleen cells as feeders. All hybrids were cloned twice to ensure stability prior to freezing cell samples or raising ascitic fluid.

**Production of ascitic fluid.** BALB/c mice primed by intraperitoneal injection of 0.5 ml of 2.6,10,14-tetramethyl-pentadecane (pristane) 1 to 4 weeks prior to injection with 10^6 selected hybridoma cells were tapped for ascitic fluid 10 days post-injection.

**Purification and characterization of monoclonal antibodies.** Purified monoclonal antibody was obtained from ascitic fluid (1 to 5 ml) by using an Affi-Gel Protein A system (MAPS; Bio-Rad Laboratories, Mississauga, Ontario, Canada) (bed volume, 3.5 ml) at 4°C. The monoclonal antibody eluted from the system was immediately dialyzed against 50 mM Tris hydrochloride-saline buffer (pH 8.0) containing 1% (wt/vol) serum albumin.

Heavy-chain-isotype analysis was performed by using *E. coli* serotype O157 LPS-coated enzyme immunoassay plates, diluted ascitic fluid, and class-specific antibody (Hybri-Clonal ELA mouse antibody screening kit; Kirkegaard and Perry Laboratories, Gaithersburg, Md.). Light chain and immunoglobulin G (IgG) subclasses were established by immunodiffusion by using 10-fold concentrations of culture supernatants and precipitating antisera specific for mouse kappa and lambda chains and IgG subclasses (Cedarlane Laboratories, Hornby, Ontario, Canada). The O antigen specificity was determined by immunodiffusion experiments employing both the alkali-treated LPS and O polysaccharide antigens of *E. coli* O157:H7 (8).

**Bacterial strains.** *E. coli* strains from the CDC culture collection belonging to several serotypes of serogroup O157 (O157:H7, O157:H16, O157:H19, O157:H45, and O157:H-), were grown on nutrient agar (Oxoid, Ltd., London, England) plates incubated for 18 to 24 h at 36°C. For determination of cross-reactions, *E. coli* O7:H4 (E.c. 389), *E. coli* O116:H10 (E.c. 158), and *Escherichia hermanii* strains from our culture collection were grown as described above.

**Antibodies.** Purified monoclonal antibodies obtained from ascitic fluids of three hybridomas, O157(1), O157(2), and O157(3), were assayed for specificity.

**Agglutination.** Agglutination assays were performed on glass slides by mixing a drop (20 μl) of serial dilutions of ascitic fluid in PBS with a loopful of bacteria. Agglutination reactions were scored within 1 min. Bacterial suspensions in 1 drop of PBS were used as controls. Agglutination reactions were determined by using live bacteria, and for confirmation, tests were repeated with the homologous bacterial suspension heated for 1 h at 100°C.

**Coagglutination.** *Staphylococcus aureus* Cowan 1 NCTC 8530 was used for the preparation of protein A cell suspension. Bacteria were grown for 18 h on Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy agar plates incubated at 36°C and collected in PBS. After three washes with PBS, bacteria were suspended in PBS–0.5% Formalin and left at room temperature with constant stirring for 3 h. Subsequently, the bacteria were washed again three times with PBS and then heated for 10 min at 80°C and washed two more times in PBS. A 10% (vol/vol) suspension was made in Tris buffer (pH 8.0) containing 0.01% sodium azide.

Affinity-purified murine immunoglobulin was coated to protein A cell suspension as described by Kromwall (22), except that the suspension buffer was Tris hydrochloride (pH 8.0) containing 1% bovine serum albumin. Murine immunoglobulin diluted 1/10 in Tris hydrochloride buffer (pH 8.0) (10 μl) was incubated with 1 ml of 10% of the protein A cell suspension for 3 h at room temperature, after which the suspension was further diluted with 4 ml of Tris hydrochloride buffer (pH 8.0) containing 1% bovine serum albumin and 0.01% sodium azide and was stored at 4°C (the final dilution of the antibody was 1/5,000).

**RESULTS AND DISCUSSION.**

Murine monoclonal antibodies to the LPS of *E. coli* serotype O157:H7 were prepared by the hybridoma technique (21). Immunization of BALB/c mice with killed, whole cells of *E. coli* O157:H7 induced a strong humoral response to the LPS antigen, and splenic lymphocytes from two mice were fused with the parental cell line Sp2/O (32). On the basis of ELISA screening using homologous purified *E. coli* O157:H7 smooth LPS, 15 clones producing specific antibody were identified. After recloning, eight cell lines were recovered, and from these, three were finally selected [O157(1), O157(2), and O157(3)] on the basis of the specificity of the secreted antibody. The heavy chain isotype and light chain class of each antibody were determined by immunoprecipitation, and the results are recorded in Table 1.

O-antigen specificity of the antibodies was established by immunodiffusion experiments employing both the alkali-
treated LPS and the O polysaccharide from E. coli serotype O157:H7. Each antibody gave a single precipitin line with the alkali-treated LPS, but only antibodies 157(1) and 157(2) precipitated with the O polysaccharide. Antibody 157(3) precipitated O polysaccharide in the presence of 3% polyethylene glycol (molecular weight, 6,000). Slide agglutination tests with suspensions of E. coli O157 cells confirmed the specificities and relative titers of the three selected monoclonal antibodies (Table 1) established earlier by ELISA titrations of ascitic fluid. These results identified antibody 157(2) as the highest-titer reagent for agglutination, and this antibody was chosen for the studies reported in this paper.

Agglutination experiments were made with ascitic fluid and a panel of E. coli serotype O157 strains (Table 2), and on the basis of these results, an ascitic fluid dilution of 1/40 was selected as the working dilution for agglutination tests performed with live or heated suspensions of bacterial cells. Known serological cross-reactions previously reported for rabbit polyclonal E. coli O157 antiserum with E. coli O7, E. coli O116 (14), and E. hermanii (2) were not observed with the monoclonal antibody 157(2), even when the ascitic fluid was used undiluted or at dilutions of 1/5 or 1/10. In addition, negative agglutination and coagglutination were obtained with strains of E. coli serotypes O1:H6, O6:H1, O6:H31, O7: H4, O7:H4, H15, O11:H16, O15:H18, O18:HNM, O20:H32, O21:H2, O26:HNM, O26:H11, O26:H32, O77:H18, O89: NM, O110:H45, O116:H10, O128:H8, O153:H25, OR:H2, OR:H33, OR:H4, and OR:NM and with Shigella flexneri, Y. enterocolitica serotype O:9, E. hermanii, and Hafnia alvei.

Although ELISA titers remained unchanged during long-term storage of ascitic fluid, agglutination titers of the protein A affinity-purified antibody stored at 4°C were found to drop from an initial 1/320 to 1/4 after 4 months of storage. Long-term storage of the antibody at 4°C was achieved by coating protein A-purified antibody to S. aureus Cowan 1 cells, and in this form the preparation was suitable for use in coagglutination reactions, maintaining its initial reactivity after storage at 4°C for over 1 year.

While the monoclonal antibody methods proved specific for the identification of the E. coli serotype O157 antigen, the positive identification of E. coli O157:H7 strains requires an additional serological identification of the H7 flagellar antigen for complete characterization.

Agglutination and coagglutination results demonstrate that the monoclonal antibody typing reagent provides the required sensitivity and specificity for identification of all E. coli serotype O157 strains. In addition, this reagent is superior to the conventional polyclonal antibody, since it is free from the serological cross-reactions which frequently give false-positive identifications.

LITERATURE CITED


