Monoclonal Antibodies for Detection of the H7 Antigen of *Escherichia coli*

Yongsheng He  
*U.S. Meat Animal Research Center, USDA-ARS, Clay Center, Nebraska*

James E. Keen  
*U.S. Meat Animal Research Center, USDA-ARS, Clay Center, Nebraska*

Ralph B. Westerman  
*U.S. Meat Animal Research Center, USDA-ARS, Clay Center, Nebraska*

E. Travis Littledike  
*U.S. Meat Animal Research Center, USDA-ARS, Clay Center, Nebraska*

Jimmy Kwang  
*U.S. Meat Animal Research Center, USDA-ARS, Clay Center, Nebraska*

Follow this and additional works at: [http://digitalcommons.unl.edu/usdaarsfacpub](http://digitalcommons.unl.edu/usdaarsfacpub)

Part of the [Agricultural Science Commons](http://digitalcommons.unl.edu/usdaarsfacpub)


[http://digitalcommons.unl.edu/usdaarsfacpub/5](http://digitalcommons.unl.edu/usdaarsfacpub/5)

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Agricultural Research Service, Lincoln, Nebraska at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Publications from USDA-ARS / UNL Faculty by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
Monoclonal Antibodies for Detection of the H7 Antigen of Escherichia coli

YONGSHENG HE,* JAMES E. KEEN, RALPH B. WESTERMAN, E. TRAVIS LITTLEDIKE,† AND JIMMY KWANG

U.S. Meat Animal Research Center, Agricultural Research Service, U.S. Department of Agriculture, Clay Center, Nebraska 68933

Received 1 March 1996/Accepted 5 July 1996

Two murine monoclonal antibodies (MAbs) (2B7 and 46E9-9) reactive with the H7 flagellar antigen of Escherichia coli were produced and characterized. A total of 217 E. coli strains (48 O157:H7, 4 O157:NM, 36 O157:non-H7, 22 H7:non-O157, and 120 non-O157:non-H7), 17 Salmonella serovars, and 29 other gram-negative bacteria were used to evaluate the reactivities of the two MAbs by indirect enzyme-linked immunosorbent assay (ELISA). Both MAbs reacted strongly with all E. coli strains possessing the H7 antigen and with H23- and H24-positive E. coli strains. Indirect ELISA MAb specificity was confirmed by inhibition ELISA and by Western blotting (immunoblotting), using partially purified flagellins from E. coli O157:H7 and other E. coli strains. On a Western blot, MAb 46E9-9 was more reactive against H7 flagellin of E. coli O157:H7 than against H7 flagellin of E. coli O1:K1:H7. Competition ELISA suggested that MAbs 2B7 and 46E9-9 reacted with closely related H7 epitopes. When the ELISA reactivities of the MAbs and two commercially available polyclonal anti-H7 antisera were compared, both polyclonal antisera and MAbs reacted strongly with E. coli H7 bacteria. However, the polyclonal antisera cross-reacted strongly both with non-H7 E. coli and with many non-E. coli bacteria. The polyclonal antisera also reacted strongly with H23 and H24 E. coli isolates. The data suggest the need to define serotype-specific epitopes among H7, H23, and H24 E. coli flagella. The anti-H7 MAbs described in this report have the potential to serve as high-quality diagnostic reagents, used either alone or in combination with O157-specific MAbs, to identify or detect E. coli O157:H7 in food products or in human and veterinary clinical specimens.

Escherichia coli O157:H7 was first identified as a human pathogen and cause of hemorrhagic colitis in the United States in 1982 (32). Many cases and outbreaks of hemorrhagic colitis and hemolytic uremic syndrome have been associated with E. coli O157:H7 infections since then (1–3, 8, 9, 15–17, 21, 31, 33, 37, 38). Food products contaminated with E. coli O157:H7 have commonly been identified as the source of infection (15, 27). Therefore, the development of reagents and methods to detect E. coli O157:H7 in food products and in clinical medicine specimens has recently been the focus of much research. While several methods have been developed to detect and isolate E. coli O157:H7 (27), there is currently no simple, rapid, and accurate technique suitable for routine use by clinical medicine or food microbiology laboratories. A major limiting factor for E. coli O157:H7 detection has been the lack of high-quality immunodiagnostic reagents.

E. coli O157:H7 bacteria contain specific O polysaccharide (O157) and flagellar protein (H7) antigens. Therefore, the production of monoclonal antibodies (MAbs) against unique epitopes on the O157 and H7 antigens is a logical approach to generate useful immunodiagnostic reagents for sensitive and specific identification of E. coli O157:H7. Polyclonal (23, 24, 36) and monoclonal (30, 35) antibodies against the O157 antigen have been produced and used to screen for E. coli O157:H7. MAbs against verotoxins VT-I and VT-II (28, 29, 34) of enterohemorrhagic E. coli and against an outer membrane protein of E. coli O157:H7 and O26:H11 have also been produced and utilized for E. coli O157:H7 detection (14, 18, 25, 26). Since many nonpathogenic E. coli O157:non-H7 isolates are known, detection of the O157 antigen independent of the H7 antigen is insufficient for E. coli O157:H7 identification. However, some studies of pathogenic E. coli have identified field isolates as belonging to the O157 serotype without determining the H serotype and assumed that the flagellar serotype was H7 (10). In many other field and clinical studies, the services of a reference laboratory were required for H serotyping, since there is currently no easy method to confirm the presence or absence of H7 on E. coli isolates. No anti-H7 MAbs are currently available to our knowledge. In this article, the production and characterization of two MAbs reactive with the H7 antigen of E. coli are described. These MAbs have the potential to be used alone or in combination with O157-specific MAbs to accurately identify E. coli O157:H7 in food and clinical specimens.

MATERIALS AND METHODS

Bacterial strains. E. coli O157:H7 strain ATCC 43895 (American Type Culture Collection, Rockville, Md.) was used for mouse immunization for hybridoma production and as the positive-control antigen for MAb screening. Additional bacteria used to characterize MAb reactivity included 47 other E. coli O157:H7 strains, 4 E. coli O157:NM strains, 23 E. coli O157:non-H7 strains, 22 E. coli H7:non-O157 strains, 73 non-O157:non-H7 E. coli strains, 17 salmonella serovars, and 29 other gram-negative bacteria. Isolates were either purchased from the American Type Culture Collection or generously provided by the Centers for Disease Control and Prevention (Atlanta, Ga.), the National Animal Disease Center (Ames, Iowa), the National Veterinary Services Laboratories (Ames, Iowa), the E. coli Reference Center (ECRC; Pennsylvania State University, University Park, Pa.), and South Dakota State University (Brookings, S.Dak.). In addition, 47 E. coli isolates representing flagellum serotypes H1 to H56 were provided by the ECRC. A detailed list of all bacterial isolates (strain, description, and source) is available upon request.

* Corresponding author. Mailing address: USDA, ARS, U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933. Phone: (402) 762-4180. Fax: (402) 762-4375. Electronic mail address: he@aux.marc.usda.gov.
† Present address: Omaha College of Health Careers, Omaha, NE 68154.
Anti-H7 polyclonal sera. Anti-H7 polyclonal rabbit sera were purchased from Difco Laboratories (Detroit, Mich.) and also provided to the ECRC.

Partial purification of *E. coli* flagellin. Bacteria were grown and selected for motility by the methods of Fujita et al. (7) with modifications. Bacterial stock in Tris-citrate buffer was inoculated into fresh broth (2% blood, 0.5% glucose; Difco Laboratories) and incubated in an orbital shaker for 6 to 8 h at 37°C. A loop of this culture was then stabbed into the center of a semisolid nutrient agar (flagellum broth-0.30% agar-8% gelatin; Difco Laboratories) plate (150 by 15 mm). Bacteria were then grown overnight at 37°C, and the plates positioned at a slight angle in the incubator. A sterile loop was used to cut and lift out a strip (5 by 10 mm) of agar at the leading edge of the bacterial growth ring on the elevated (antigravity) edge of the plate. This agar strip containing heavy bacterial growth was placed into flagellum broth and incubated for 1 h at 37°C. The bacteria were harvested by centrifugation at 15,000 × g for 1 h at 4°C, and the cell pellet was suspended in ice-cold 20 mM Tris buffer, pH 7.4. The cell suspension was then homogenized twice in a Waring blender (to remove flagellar filaments from the cell surface) for 2.5 min on ice, with a 5-min rest in between. The resultant homogenate was centrifuged at 10,000 × g for 30 min at 4°C, and the supernatant was collected. The flagellar proteins in the supernatant were then pelleted at 100,000 × g for 3 h at 4°C. The pellet was resuspended in phosphate-buffered saline (PBS) and stored at −20°C until use.

Whole-bacterium antigen preparation. *E. coli* was grown in flagellum broth and then on semisolid nutrient agar, as described above. Bacteria from the leading growth edge on the elevated side of the nutrient agar plate were inoculated into flagellum broth and incubated overnight at 37°C. This culture was then used to inoculate the entire surface of Trypticase soy agar plates. After overnight incubation at 37°C, the bacteria from each plate were loop harvested, diluted in 1 ml of deionized distilled water, heat inactivated (1 h at 60°C), and sonicated on ice (1-min rest between strokes). Non-*E. coli* cells were then plated on Trypticase soy agar, incubated overnight at 37°C, loop harvested, diluted in 1 ml of deionized distilled water, heat inactivated, and sonicated. Whole-bacterium antigens were stored frozen at −20°C until use.

Immunization of mice. The whole-bacterium antigen suspension of *E. coli* O157:H7 ATCC 43895 was mixed with adjuvant (monophosphoryl lipid A and trehalose dicyclohexylammonium emulsion; Ribnu ImmunoChem Research Inc., Hamilton, Mont.) (20 µg/0.2 ml) according to the manufacturer's protocol. Four BALB/c mice (about 8 weeks old) were intraperitoneally (i.p.) injected with 0.2 ml of the bacterial-antigen emulsion. The mice were boosted with partially purified flagellin (20 µg per mouse) of *E. coli* O157:H7 without adjuvant by three i.p. injections over a 2-week interval. The final i.p. injection of the partially purified flagellin (20 µg per mouse) was given 4 days before fusion.

Fusion and hybridoma production. Fusion and hybridoma production were performed by the methods of Hockfield et al. (13). Spleen cells from an immunized mouse were fused with X63Ag8-653 mouse myeloma cells (American Type Culture Collection) in serum-free Dulbecco's modified Eagle medium (Gibco BRL, Grand Island, N.Y.) by using 50% polyethylene glycol (molecular weight, 1,450) (Sigma Chemical Company, St. Louis, Mo.). Fused cells were resuspended in hypoxanthine-aminopterin-thymidine medium (Dulbecco's modified Eagle medium; 100 µg/ml hypoxanthine; 100 µg/ml aminopterin; 10 µg/ml thymidine; 10% mouse macrophage cell cultured medium; 10% hybridoma cloning factor [Fisher Scientific, St. Louis, Mo.], 15% fetal bovine serum, antibiotics, and hypoxanthine-aminopterin-thymi- dine medium).

Cloning and subcloning of hybridomas. Mouse peritoneal macrophages were harvested by peritoneal lavage (13). The central 60 wells of 96-well plates (Costar, Cambridge, Mass.) were filled with hypoxanthine-aminopterin-thymi- dine medium containing the macrophages (5 × 105 per well) and cultivated for 1 to 2 days in a 37°C incubator with 8% CO2. The hybridoma cells were added to the macrophage feeder layers and cultured in the 8.6% CO2 incubator for about 2 weeks at 37°C, until the clones were large enough for ELISA screening. Hybridomas with desirable reactivity on screening by ELISA were subcloned two or three times by limiting dilution at 0.5% cell well (13).

Ascites fluid ascites. A group of retired breeder BALB/c mice was given 0.1 ml pristane (Sigma Chemical Company) by i.p. injection. After a 5- to 7-day rest period, the mice were injected with 1.0 ml of the hybridoma cell suspension (107 cells) in serum-free Dulbecco's modified Eagle medium by i.p. injection. Ascites fluid containing the MAbs was collected beginning 1 week after the cell injection.

MAb Isotyping. An antibody isotyping kit (Calbiochem-Novabiochem Corporation, La Jolla, Calif.) was used with minor modifications. Enzyme-linked immunosorbent assay (ELISA) microtiter plates were coated with rabbit antibodies against each mouse immunoglobulin (Ig) isotype for 2 h at 37°C, and culture medium or diluted ascites fluid was then added to the plate wells. After the plates were incubated and washed, peroxidase-labeled antibodies to mouse IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM were added to the wells, and the samples were reacted with substrates as described under "Indirect ELISA."

Western blotting assay. Unlabeled MAbs was serially twofold diluted with PBS and incubated for 1 h at 37°C on an ELISA plate coated with partially purified *E. coli* O157:H7 flagellin (10 µg/ml, 100 µl per well). After aspiration and washing (as described under "Indirect ELISA"), the plate was incubated for 10 min at 37°C. The rest of the procedures were followed as described under "Indirect ELISA."

Production of MAbs. Approximately 3,000 hybridoma clones resulted from one fusion; of these, 320 clones showed positive reactions to *E. coli* O157:H7 (OD > 0.2) and negative reactions to *E. coli* O78:H11 (OD < 0.05) by indirect ELISA on initial screening. Subsequent ELISA screening with 168 other bacteria identified two clones (2B7 and 46E9-9) which were reactive against the *E. coli* H7 antigen. Both MAbs 2B7 and 46E9-9 were identified as IgG1 isotype.

Specificities of MAbs. After cloning, the MAbs in hybridoma supernatant (culture media) or in diluted (1:1,000) ascites fluid were tested against a large panel of gram-negative bacteria. MAbs were tested by indirect ELISA as summarized in Table 1. Both MAbs reacted strongly with 48 of 48 *E. coli* O157:H7 isolates, 22 of 22 *E. coli* H7/non-O157 isolates, 2 of 2 *E. coli* O45:H23 isolates, and 2 of 2 *E. coli* O51:H24 isolates. Neither MAb reacted with any of the following bacteria: four *E. coli* O157:NM isolates, 23 *E. coli* O157:non-H7 isolates, 69 *E. coli* non-O157:non-H7 isolates, 17
Salmonella serovars, and 29 other gram-negative bacteria. The comparative ELISA reactivities of the two MAbs (in diluted [1:1,000] ascites fluid) and the two commercial H7 polyclonal antisera against various whole-bacterium antigens are shown graphically in Fig. 1. Both polyclonal anti-H7 sera reacted with all 48 O157:H7 and 22 H7-non-O157 E. coli strains. However, unlike the MAbs, the polyclonal sera also reacted strongly in ELISA with many non-H7 E. coli and non-E. coli bacteria. Dilution of polyclonal sera reduced this cross-reactivity but also simultaneously decreased polyclonal reactivity with H7-positive E. coli strains (data not shown).

Specificities of the MAbs were further examined with partially purified E. coli flagellins and E. coli O157 lipopolysaccharide (LPS). ELISA plates were coated with partially purified E. coli flagellins (10 μg/ml) from E. coli O157:H7, O1:K1: H7, O45:H23, O51:H24, and O157:H52; whole-bacterium antigens (50 μg/ml) from E. coli O157:H7 and O78:H11; and phenol-water-purified LPS from E. coli O157:H7. Indirect ELISA reactivities of the MAbs against these different bacterial components were then compared. As shown in Fig. 2, MAbs 2B7 and 46E9-9 reacted similarly with E. coli O157:H7 whole-bacterium antigens and with the partially purified flagellins of E. coli O157:H7, O1:K1:H7, O45:H23, and O51:H24. However, neither MAb 2B7 nor MAb 46E9-9 reacted with E. coli O157:H52 flagellin or O157:H7 LPS. As an MAb negative

**TABLE 1. ELISA reactivities of MAbs 2B7 and 46E9-9 to 216 whole-bacterium antigens**

<table>
<thead>
<tr>
<th>Bacterium(-a)</th>
<th>No. positives*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>O157:H7</td>
<td>48</td>
</tr>
<tr>
<td>O157:NM</td>
<td>0</td>
</tr>
<tr>
<td>H7:non-O157</td>
<td>0</td>
</tr>
<tr>
<td>O157:non-H7</td>
<td>22</td>
</tr>
<tr>
<td>Non-O157:non-H7</td>
<td>0</td>
</tr>
<tr>
<td>O45:H23</td>
<td>2</td>
</tr>
<tr>
<td>O51:H24</td>
<td>2</td>
</tr>
<tr>
<td>Salmonella</td>
<td>0</td>
</tr>
<tr>
<td>Others*</td>
<td>2</td>
</tr>
<tr>
<td>Other gram-negative bacteria</td>
<td>0</td>
</tr>
</tbody>
</table>

a OD at 405 nm > 0.100. The two MAbs produced the same results.

b S. arizonae, S. choleraesuis, S. dublin (n = 3), S. enteritidis, S. godesberg type O:30, S. landau, S. mbandaka (n = 3), S. newport, S. soerengae, S. typhimurium (n = 2), S. urbana type O:30, and one untypeable Salmonella sp.

c Actinobacillus suis, Aeromonas hydrophila, Alcaligenes faecalis, Bordetella bronchiseptica, Brucella abortus (n = 3), Cedecea lapagei, Citrobacter freundii, Escherichia hermannii, Edwardsiella tarda, Enterobacter cloacae, Haemophilus somnus, Hafnia alvei, Kibesliella pneumoniae, Lampropedia grimmii, Pasteurella haemolytica, Pasteurella multocida (n = 2), Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa, Pseudomonas maltophilia, Serratia marcescens, Shigella sonnei, Yersinia enterocolitica (n = 3), Yersinia pseudotuberculosis.

**FIG. 1.** Comparative ELISA reactivities of two anti-H7 MAbs and two commercial anti-H7 polyclonal antisera to different bacterial antigens. ELISA plates were coated with whole-bacterium antigens of bacteria (50 μg/ml, 100 μl per well). The diluted murine ascites fluid of MAbs 2B7 and 46E9-9 (1:1,000) and the rabbit anti-H7 antiserum from ECRC (1:2,000) and Difco Laboratories (1:8,000) were incubated with the antigens (100 μl/well). HRPO-conjugated antibodies to mouse IgG and IgM and to rabbit IgG (KPL) were diluted at 1:2,000. The data are means with standard deviations. * see Table 1.
control, an anti-O157 LPS-specific MAb (MAb 51B2) was included in these ELISAs. MAb 51B2 reacted strongly only with E. coli O157 LPS, E. coli O157:H7 whole-bacterium antigens, and partially purified E. coli O157:H7 flagellin. Neither MAb reacted with the whole-bacterium antigen from E. coli O78: H11 (negative-control antigen).

Antigen specificities of the MAbs were studied by inhibition ELISA. If the inhibiting antigen shares the epitope with the target antigen, the incubation of the inhibiting antigen with the MAb should decrease the capability of the MAb to bind to the target antigen. As shown in Fig. 3, E. coli O157:H7 flagellin displayed the highest inhibition of the binding capability of MAbs 2B7 and 46E9-9. Flagellins of E. coli O1:K1:H7, O45: H23, and O51:H24 exhibited similar levels of inhibition of the binding of MAb 2B7 to E. coli O157:H7. The flagellin of E. coli O1:K1:H7 had greater inhibitory effect than flagellins of E. coli O45:H23 and O51:H24 on the binding of MAb 46E9-9 to E. coli O157:H7. All inhibition reactions showed concentration dependence. The flagellins of E. coli O157:H16, O157:H52, and O78:H11 had no inhibitory effect on the binding activities of the MAbs to E. coli O157:H7 antigens.

Immunoblotting confirmed the specificities of MAbs 2B7 and 46E9-9, as shown in Fig. 4. The same amounts (2 μg) of partially purified flagellins from E. coli O157:H7, O157:H16, O1:K1:H7, O45:H23, O51:H24, and O157:H52 and whole-bacterium antigens (12.5 μg) of E. coli O157:H7, O78:H11, and O157:NM were loaded in three identical SDS-polyacrylamide gels. The protein profile of the different preparations in the discontinuous SDS-polyacrylamide gel was revealed by Coomassie brilliant blue staining (Fig. 4A). The H7 antigens from E. coli O157:H7 and O1:K1:H7 (lanes 1 and 3), H23 (lane 4), and H24 (lane 5) had the same molecular masses of approximately 65 kDa. H52 (lane 6) and H16 (lane 2) had molecular masses of about 63 and 56 kDa, respectively. MAb 2B7 (Fig. 4B) reacted with the purified flagellins of E. coli O157:H7 and O1:K1:H7 (lanes 1 and 3), H23 (lane 4), and H24 (lane 5), as well as the E. coli O157:H7 whole-bacterium antigen (lane 7), but did not react with H16 (lane 2) and the other two bacterial (O78:H11 and O157:NM) antigens (lanes 8 and 9). MAb 2B7 appeared to have a stronger reaction to the purified flagellin of E. coli O157:H7 than other reactive flagellins. Interestingly, MAb 46E9-9 (Fig. 4C) appeared much more reactive on Western blot to the H7 antigen from E. coli O157:H7 (lanes 1 and 7) and showed only weak reactions to H7 from E. coli O1:K1: H7, H23, and H24 (lanes 3 to 5). At least two bands were visible (63 and 65 kDa) in the E. coli O157:H7 whole-bacterium antigen preparation (lane 7), detected by MAbs 2B7 and 46E9-9 (Fig. 4B and C), with a stronger reaction occurring to the lower-molecular-weight band (63 kDa). However, only the higher-molecular-weight band (65 kDa) (lane 1) was visible when the partially purified flagellin of E. coli O157:H7 was the antigen.

Competition ELISA permitted study of epitope specificities of the anti-H7 MAbs. Preincubation of a MAb with the target antigens before the test MAb will decrease binding of the test MAb to the antigens. This results in lower ELISA OD values in the presence of competition if both MAbs react with the same or closely related epitopes. As shown in Fig. 5, the 50% homologous competition dose of MAbs 2B7 and 46E9-9 was not reached.
about 30 μg/ml. Heterologous competitions between 2B7 and 46E9-9 were very similar to the homologous competitions.

**Specificity comparison of the MAbs and polyclonal anti-H7 sera against E. coli H serotypes.** A panel of E. coli strains representing 47 different H serotypes (from H1 to H56), in addition to 18 E. coli O157:H7 isolates and 11 H7 non-O157 strains, were used to compare the ELISA reactivities of the two MAbs and two polyclonal antisera. As shown in Fig. 6, MAb 2B7 reacted strongly with all E. coli isolates possessing H7 and did not react with any non-H7 antigens, except for H23 and H24. The reactivity pattern of MAb 46E9-9 was very similar to that of MAb 2B7 (data not shown). Polyclonal antisera from ECRC and Difco Laboratories both also reacted with all E. coli isolates possessing H7. However, both polyclonal antisera, in particular the serum from Difco Laboratories, cross-reacted with many non-H7 E. coli isolates. In fact, as shown in Fig. 6, the ELISA cross-reactions of the polyclonal antisera to some non-H7 E. coli isolates were even greater than their reactions to H7 (e.g., antisera against H6 and H8 [Difco Laboratories]). Both polyclonal anti-H7 sera, like the MAbs, also strongly reacted with E. coli H23 and H24 bacteria.

**DISCUSSION**

This report describes the production and characterization of two MAbs which are reactive against the E. coli H7 flagellar antigen. A wide range of antigen preparations and techniques were used first to select anti-H7 MAbs and then to define their specificity. First, MAb reactivities against the E. coli H7 antigen were determined by indirect ELISA using whole-bacterium antigens. Of 216 whole-bacterium preparations, MAbs 2B7 and 46E9-9 were found to react strongly only to E. coli H7, H23, and H24. The H7, H23, and H24 flagellin specificity of the two MAbs was confirmed by indirect ELISA using partially purified E. coli flagellins. The inhibition ELISA showed that the partially purified H7 flagellin of E. coli O157:H7 efficiently blocked binding of MAb 2B7 and MAb 46E9-9 to E. coli O157:H7 whole-bacterium antigens. In addition, the flagellins...
noblots were run under protein-reducing conditions, while only with H7 from O157:H7 or O1:K1:H7 by indirect ELISA but reacted strongly with MAb2B7. Interestingly, MAb2B7 reacted strongly with visualization of the antigenic specificities of the two MAbs. MAb46E9-9 and H7 antigens from either O157:H7 or O1:K1:H7 by indirect ELISA and Western blots. On the other hand, MAb46E9-9 reacted strongly with E. coli H23, H24, and H7 antigens from O157:H7 or O1:K1:H7 by indirect ELISA but reacted strongly only with H7 from E. coli O157:H7 on Western blot. Immunoblots were run under protein-reducing conditions, while ELISAs were not. These findings suggest that MAb 46E9-9 may react with an epitope unique to the H7 antigen of E. coli O157:H7, the nucleic acid and amino acid sequences of the H7 antigens of E. coli O157:H7 and O1:K1:H7 may not be identical, and the H7 epitope of E. coli O1:K1:H7 and the H23 and H24 epitopes with which MAb 46E9-9 reacts may be conformational and not linear.

Serotyping of O and H antigens by agglutination using panels of individual or pooled preabsorbed polyclonal antisera has been used to classify pathogenic E. coli and other enterobacteria for many years. However, the inadequacies of polyclonal antisera for serotyping some enterobacteria have been reported (5). MAbs are not currently used for E. coli serotyping in reference laboratories such as the ECRC but have the potential to serve as higher-quality immunodiagnostic reagents as shown in this report. To our knowledge, only two polyclonal anti-H7 sera are available commercially in the United States; these antisera are used in several applications, including the confirmatory H serotyping test for E. coli O157:H7 (36) and motility determination for E. coli H7 isolates (6). On the basis of the indirect ELISA technique described in this report, the anti-H7 MAbs 2B7 and 46E9-9 are much more specific than these two polyclonal anti-H7 antisera as measured against a wide range of bacteria including E. coli O157:H7, E. coli non-O157:non-H7, Salmonella spp., and other gram-negative bacteria. MAbs 46E9-9 and 2B7 reacted against E. coli H7 without cross-reactivity to other bacteria except for E. coli O45:H23 and O51:H24. In contrast, the anti-H7 serum from Difco Laboratories at a 1:8,000 dilution and the preabsorbed anti-H7 serum from the ECRC at a 1:2,000 dilution cross-reacted with many non-O157:non-H7 E. coli isolates and even some non-E. coli bacteria in the ELISA (Fig. 1). The patterns of lack of MAb cross-reactivity and common polyclonal antisera cross-reactivity were repeated when the antibodies were tested against an antigen panel of 48 E. coli H serotypes (H1 to H56) (Fig. 6). In addition to higher specificity, the MAbs also offer the potential of a more standardized typing reagent compared with polyclonal antisera.

Cross-reactivity makes differentiation among the H7, H23, and H24 E. coli serotypes difficult whether polyclonal antisera or our MAbs are used. Subtle relative differences in the microagglutination reactions between H7, H23, and H24 polyclonal antisera and the bacterial antigen are used by the ECRC to serotype isolates among these three flagellar variants (39). MAbs 2B7 and 46E9-9 reacted strongly with E. coli O45:H23 and O51:H24 in our indirect ELISA, using either whole bacterial antigens or partially purified flagellins, as shown in Fig. 1, 2, and 6. The two polyclonal anti-H7 antisera also reacted strongly with the H23 and H24 bacteria, as indicated in Fig. 1 and 6. However, inhibition ELISA and Western blot reactions for MAb 46E9-9 (Fig. 3 and 4C) were strongest with H7 from E. coli O157:H7 and weaker with H7 from O1:K1:H7 (the reference H7 strain), H23, or H24. These data suggest that MAb46E9-9-defined differences may exist in the flagellin structure among H7 isolates and between H7 and H23 or H24. Since H antigenicity is dependent on the amino acid sequence of the component flagellin protein, only minor nucleic or amino acid compositional differences among E. coli H7, H23, and H24 may define the specific serotypes. Our laboratory recently compared flagellin gene and protein sequences between H7 from E. coli O157:H7 and H23 from E. coli O45:H23 (19). The H7 antigenic domain was localized to a 23-amino-acid sequence in the central variable region of the flagellin gene. A single nucleotide difference between H7 and H23 was found in this region, resulting in one amino acid substitution. Additional comparative flagellin sequence and epitope map-
ping among various H7 isolates, between H7 and H24 strains and between H23 and H24 strains, may permit precise definition of these E. coli H antigen serotypes. In addition to serotyping, E. coli flagella have also been classified by surface ultrastructural morphology into six principal groups (i.e., morphotypes A to F) (20). Interestingly, both E. coli H7 and H23 belong to the same morphotype (E), while H24 belongs to morphotype C.

In conclusion, we have produced and characterized two MAbs directed against the H7 flagellar antigen of E. coli. These unique MAbs, whether used alone or in combination with anti-O157-specific MAbs (which were also produced in our laboratory), could be used for detection of the highly pathogenic E. coli O157:H7 serotype in applications ranging from meat or food inspection to medical specimen examination and research.

ACKNOWLEDGMENTS

We thank Richard A. Wilson of the ECRC for generously providing numerous bacterial strains and for helpful suggestions. We also acknowledge the excellent technical assistance of Sandy Fryda-Bradley, Ron Mlejnek, Tammy Sorensen, and Nancy Ferrell and thank Joan Rosch for careful preparation of the manuscript.

REFERENCES


FIG. 6. Comparison of ELISA reactivities of MAb 2B7 and two commercially available polyclonal anti-H7 antisera against E. coli serotypes. ELISA plates were coated with whole-bacterium antigens (50 μg/ml) of E. coli O157:H7, H7:non-O157, and 47 other H antigen serotypes. Diluted ascites fluid of MAb 2B7 (1:1,000), preabsorbed rabbit polyclonal anti-H7 serum from ECRC (1:2,000), and polyclonal rabbit anti-H7 serum from Difco Laboratories (1:8,000) were incubated in duplicate wells with the antigens for 10 min at 37°C. After washing, HRPO-conjugated rabbit anti-mouse IgG and IgM antibody (KPL; 1:2,000) was added to MAb 2B7 plates and HRPO-conjugated goat anti-rabbit IgG antibody (KPL; 1:2,000) was added to polyclonal antibody plates. ELISA reactivity is expressed as mean OD (405 nm) of duplicate wells using the ABTS substrate.
infection in Minnesota child day-care facilities. JAMA 269:883–888.


