Murine Monoclonal Antibodies Specific for Lipopolysaccharide of *Escherichia coli* O26 and O111

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Murine Monoclonal Antibodies Specific for Lipopolysaccharide of Escherichia coli O26 and O111

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Monoclonal antibody (MAb) 12F5 reacted with 35 Escherichia coli O26 isolates and cross-reacted with 1 of 365 non-E. coli O26 isolates. MAb 15C4 reacted with 30 E. coli O111 strains and 8 Salmonella O35 strains (possessing identical O antigen) but not with 362 other bacterial strains. Lipopolysaccharide immunoblots confirmed MAb O-antigen specificity.

Escherichia coli O26 and O111 are classic enteropathogenic and emergent enterohemorrhagic E. coli (EHEC) serotypes. Among the 13 enteropathogenic serogroups, E. coli O26 and O111 are epidemiologically predominant and major causes of pediatric diarrhea, especially in developing countries (22, 29). E. coli O111 caused 28% of the 50 U.S. infantile diarrhea outbreaks from 1934 to 1987 (33). Over the past 20 years, Shiga toxin-producing O26 and O111 emerged worldwide as the clinically most important non-O157 EHEC serotypes and were linked with sporadic cases and outbreaks of human disease, including hemorrhagic colitis and hemolytic-uremic syndrome (HUS) (8, 9, 28). Several EHEC O26- and O111-caused outbreaks have occurred in Europe and Australia, and 25% of North American HUS cases may be attributable to non-O157 EHEC (13). In the United States, EHEC O111:NM caused a family cluster of diarrhea and HUS (3), and EHEC O111:H8 caused a gastroenteritis outbreak affecting more than 50 persons (1).

Pathogenic E. coli O26 and O111 are typically identified by agglutination, using absorbed polyclonal antibodies (PAb) against lipopolysaccharide (LPS) O antigen generated by immunizing rabbits with E. coli reference strains (22, 26). However, absorbed anti-E. coli O26 PAb may cross-react with E. coli possessing O antigens 4, 13, 25, 32, 100, and 102 (26) and with Pseudomonas aeruginosa O12 (34). Although no E. coli O antigens cross-react with absorbed anti-E. coli O111 PAB (26), this PAB strongly agglutinates Salmonella O35 isolates (12, 24) because of the identical chemical structure of the E. coli O111 and Salmonella O35 O antigens (4, 14, 25). Monoclonal antibodies (MAb) reactive with E. coli O26 (15, 27) and O111 (2, 5–7, 21, 23) have been reported but were not characterized for diagnostic sensitivity and specificity. The public-health importance and prevalence of pathogenic E. coli O26 and O111 in animals, humans, and foods are probably underestimated due to the lack of available specific serotyping reagents and because most clinical laboratories do not routinely serotype fecal E. coli isolates (30). We generated MAb against E. coli O26 and O111 in order to have accurate serotyping reagents for use in planned epidemiologic surveys of non-O157 EHEC occurrence in livestock.

MAb were produced from splenocytes of BALB/c mice immunized with E. coli O26:H11 (ECRC DEC 10A) or O111:NM (ECRC 95.0122) whole-bacterium antigen. Immunization, hybridoma and ascites production, and MAb screening and characterization protocols were previously described (10, 11). MAb were isotyped with a commercial kit (Zymed Laboratories, Inc., South San Francisco, Calif.). One anti-O26 MAb (12F5) and one anti-O111 MAb (15C4) were generated and characterized. MAb diagnostic sensitivity and specificity were estimated by enzyme-linked immunosorbent assay (ELISA) reactivity with whole-bacterium lysates from 400 gram-negative bacterial strains: 35 E. coli O26 strains, 30 E. coli O111 strains, 26 non-E. coli O26 strains reported to react with anti-O26 PAb (E. coli O4 [n = 14], O13, O25 [n = 6], O32, O100, and O102; P. aeruginosa O12 [n = 2]), 225 other E. coli strains of various O and H serotypes, 57 Salmonella serovars (including O35 [n = 8]), and 27 other gram-negative bacterial strains. For ELISA, bacterial-antigen-coated plates were sequentially incubated with MAb (diluted ascites fluid), horseradish peroxidase (HRP)-conjugated antibody against mouse immunoglobulin G (IgG) plus mouse IgM (anti-mouse IgG plus mouse IgM, and 2,2’-azino-di-[3-ethylbenzthiazoline sulfonate] solution (ABTS peroxidase substrate) (10). ELISA optical density was measured at dual wavelengths of 405 and 490 nm (OD405/490); and OD405/490 of ≥0.200 was considered positive. Dot box plots (16) of MAb ELISA OD405/490 values for bacterial-antigen subsets were generated (Prism 3.0; Graph Pad Software Inc., San Diego, Calif.), and MAb diagnostic-sensitivity and -specificity point estimates with exact binomial 95% confidence intervals (CI) were calculated (Epi Info 6.0; Centers for Disease Control and Prevention, Atlanta, Ga.). Sensitivity was defined as the number of MAb ELISA-positive isolates per the total number of isolates tested possessing the target (O111 or O26) antigen. Specificity was defined as the number of MAb ELISA-nonreactive isolates per the total number of isolates tested that did not possess the target antigen.

MAb 12F5 (IgM isotype) reacted strongly by ELISA with 35 E. coli O26 isolates (sensitivity, 100%; 95% CI of 90.0 to 100) and cross-reacted with 1 of 369 non-E. coli O26 isolates (specificity, 99.73%; 95% CI of 98.5 to 99.99) (Fig. 1). The cross-reactive strain, E. coli O4:NM (CDC3377-85), was derived from a sporadic hemorrhagic-colitis case (32) and was subsequently retyped by the E. coli Reference Center (ECRC), Pennsylvania State University, University Park, as O negative:NM; the original O4 antigen was apparently lost on passage since its 1983 isolation. MAb 12F5 was later found to cross-react with bovine E. coli O-negative:NM field isolates (J. Keen, unpublished data). While the E. coli O26 O-antigen structure is known (20), the nature of the MAb-defined O-antigen
epitope common to E. coli O26 and E. coli O-negative:NM isolates is unknown. Importantly, MAAb 12F5 did not react by ELISA with any bacteria that cross-react with anti-O26 PAb by agglutination.

MAAb 15C4 (IgG3 isotype) reacted strongly by ELISA with 30 E. coli O111 strains (sensitivity, 100%; 95% CI of 88.4 to 100) and 8 Salmonella O35 isolates (Fig. 2). MAAb interaction with Salmonella O35 represents true antibody-antigen reactivity, not cross-reactivity, since the E. coli O111 and Salmonella O35 O antigens are identical (14). MAAb 15C4 did not react with 317 non-E. coli O111 or 49 Salmonella non-O35 isolates (specificity, 100%; 95% CI of 99.0 to 100).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (18), followed by Western immunoblotting (31) using semipurified LPS (17, 19) and whole-bacterium lysates from E. coli O26 and O111 and non-E. coli O26 and O111 as antigens, confirmed MAAb O-antigen specificity. Reactive bands from gels transferred onto polyvinylidene difluoride membranes and incubated with MAAb were revealed with HRP-conjugated rabbit anti-mouse IgG + IgM and dianminobenzidine substrate (10).

FIG. 1. Dot box plots of ELISA reactivities of 343 whole-bacterium lysates with anti-E. coli O26 MAAb 12F5. ELISA plates coated with whole-bacterium antigens were incubated sequentially with MAAb 12F5 (ascites fluid, 1:7,000), anti-mouse IgG + IgM-HRP, and ABTS solution. A MAAb ELISA OD_{405nm} of >0.200 was considered positive. Dots represent means of duplicate OD values. The bottom and top edges of the superimposed box plots are the 25th and 75th distribution percentiles, respectively; the central horizontal line is the median (50th percentile), and the central vertical lines extend from the box as far as the data extend (range). CRX, non-E. coli O26 bacteria known to cross-react with anti-E. coli O26 PAb. Data for MAAb ELISA reactivity with 57 Salmonella isolates are not shown.

FIG. 2. Dot box plots of ELISA reactivities of 351 whole-bacterium lysates with anti-E. coli O111 MAAb 15C4. ELISA plates coated with whole-bacterium antigens were incubated sequentially with MAAb 15C4 (ascites fluid; 1:64,000), anti-mouse IgG + IgM-HRP, and ABTS solution. OD values represent means of duplicate wells. For dot box plot interpretation, see the legend for Fig. 1. Data for MAAb ELISA reactivity with 49 non-O35 Salmonella isolates are not shown.
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![FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blots of MAb 12F5 (anti-*E. coli* O26) and 15C4 (anti-*E. coli* O111) on purified LPS. Lanes 1, *E. coli* O26:H11 H311b; lanes 2, *E. coli* O26:H11 DEC 10A; lanes 3, *P. aeruginosa* O12; lanes 4, *E. coli* O4:NM CDC3377-85; lanes 5, *E. coli* O4:H5 U4-41; lanes 6, *E. coli* O111:NM Stoke W; lanes 7, *E. coli* O111:NM ECRC 95.1022; lanes 8, *Salmonella* serovar Adelaide O35 ATCC 10718. After electrophoresis, one gel was silver stained (A). The other two gels were transferred onto polyvinylidene difluoride membranes and incubated with cell culture antiserum. Molecular mass (in kilodaltons) is indicated on the left.

The ladder pattern characteristic of LPS was observed on silver-stained gels and MAb-incubated membranes (Fig. 3). MAb 12F5 reacted with LPS from *E. coli* O26:H11 and O4:NM CDC3377-85 but not with LPS from five other bacterial strains. MAb 15C4 reacted only with *E. coli* O111 and *Salmonella enterica* serovar Adelaide O35 LPS. Similar immunoblots resulted when whole-bacterium lysates were used in place of LPS (data not shown).

In summary, we produced and characterized MAb against *E. coli* O26 and O111 that were sensitive and specific as assessed by ELISA against 400 target and nontarget isolates. Immunoblots against purified LPS confirmed MAb O-chain specificity. These MAb have potential use as immunodiagnostic reagents whether used alone or in combination with other phenotypic or genotypic markers.

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