Immunological Characterization of *Escherichia coli* O157:H7 Intimin γ1

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Portions of the intimin genes of *Escherichia coli* O157:H7 strain E319 and of the enteropathogenic *E. coli* O127:H6 strain E2348/69 were amplified by PCR and cloned into pET-28a(+) expression vectors. The entire 934 amino acids (aa) of *E. coli* O157:H7 intimin, the C-terminal 306 aa of *E. coli* O157:H7 intimin, and the C-terminal 311 aa of *E. coli* O127:H6 intimin were expressed as proteins fused with a six-histidine residue tag (six-His tag) in pET-28a(+). Rabbit antisera raised against the six-His tag–full-length *E. coli* O157:H7 intimin protein fusion cross-reacted in slot and Western blots with outer membrane protein preparations from the majority of enterohemorrhagic and enteropathogenic *E. coli* serotypes which have the intimin gene. The *E. coli* strains tested included isolates from humans and animals which produce intimin types α (O serogroups 86, 127, and 142), β1 (O serogroups 5, 26, 46, 69, 111, 126, and 128), γ1 (O serogroups 55, 145, and 157), γ2 (O serogroups 111 and 103), and ε (O serogroup 108) and a non-typeable intimin (O serogroup 80), results based on intimin type-specific PCR assays. Rabbit antisera raised against the *E. coli* O157:H7 C-terminal fusion protein were more intimin type-specific than those raised against the full-length intimin fusion protein, but some cross-reaction with other intimin types was also observed for these antisera. In contrast, the monoclonal antibody Inty1.C11, raised against the C-terminal *E. coli* O157 intimin, reacted only with preparations from intimin γ1–producing *E. coli* strains such as *E. coli* O157:H7.

*Escherichia coli* O157:H7 is associated with hemorrhagic colitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome in humans (21, 22). In addition to producing Shiga-like (Vero) toxin and enterohemolysin (6), *E. coli* O157:H7 has been shown to attach to the cytoplasmic membranes of intestinal epithelial cells, to efface their microvilli, and to cause actin to accumulate beneath sites of bacterial membranes of intestinal epithelial cells, to efface their microvilli, and to cause actin to accumulate beneath sites of bacterial attachment (8). These features are shared with several other enterohemorrhagic *E. coli* (EHEC) serotypes and members of the enteropathogenic *E. coli* (EPEC) group (23, 24, 30).

The eae gene, which has been shown to be necessary for attaching and effacing activity, encodes a 94- to 97-kDa outer membrane protein (OMP) which is termed intimin (20). This gene is located in a chromosomal pathogenicity island also known as the locus of enterocyte effacement (LEE) (9). The entire nucleotide sequences of the 35-kbp LEE of EPEC strain E2348/69 (O127:H6) and the 43-kbp LEE of *E. coli* O157:H7 strain EDL933 have been determined (11, 36, 46). In addition to encoding intimin, the LEE encodes a number of other proteins which are necessary for intimate attachment of these bacteria to epithelial cells, such as proteins which are part of a type III secretion system (Sep and Esc proteins), the translocated intimin receptor (Tir), CesT (a Tir chaperone protein), and *E. coli* secreted proteins (EspA, EspB, and EspD).

Analysis of the nucleotide sequences of the intimin genes from different EHEC and EPEC strains has shown a high degree of homology in the 5′-two-thirds of the genes and a significant degree of heterogeneity in the 3′-one-third of the genes (2, 5, 20, 26, 33, 35, 45). Gannon et al. (16) identified five variants of the eae gene in *E. coli* strains from human and animal sources by examining the restriction fragment length polymorphisms (RFLP) of PCR products obtained from the amplified 5′ conserved region of the gene. Similarly, Boerlin et al. (7) identified six variants of this *E. coli* gene by another RFLP-PCR approach, and Wieler et al. (45) identified four variants of the gene in Shiga-like-toxin-producing *E. coli* strains of bovine origin by intimin type-specific (TS) PCR assays. Adu-Bobie et al. (1) reported five distinct types of intimin among the *E. coli* strains based on TS-PCR assays that used oligonucleotide primers complementary to the 3′ end of specific intimin genes. They noted that among strains of the two phylogenetically defined subgroups of EPEC (clones 1 and 2), clone 1 strains produce a common intimin type, which they designated α, and clone 2 strains produce another common intimin type, which they designated β. They further noted that two strains of *E. coli* O86:H34 produce a distinct intimin type, which they designated δ, and that *E. coli* O157:H7 (H1) and EPEC O55:H7 strains produce a common and distinct intimin type, which they designated γ. Reid et al. (37) have also described a multiplex PCR assay for differentiation among intimin types α, β, and γ, which is in agreement with the typing scheme of Adu-Bobie et al. (1).

Recently, Oswald et al. (33) have described a TS-PCR assay which identifies a fifth intimin variant, from *E. coli* serotype O103:H2, which they have called intimin ε. They have also reclassified certain intimin types based on the degree of nucleotide sequence homology and on RFLP-PCR profiles. In their scheme, intimin γ is divided into two subtypes, with intimin γ1 present in strains of EHEC serotypes O145:H− and O157:H7(H−) and EPEC serotype O55:H7 and intimin γ2 present in strains of EHEC serotypes O86:H40 and O111:H8(H−) and...
EPEC serotypes O127:H40 and O128:H8(H −). Furthermore, the single representative of the intimin δ group (EPEC O86: H34) reported by Adu-Bobie et al. (1) was reclassified as intimin β2 by Oswald et al. (33) based on the similarity of the nucleotide sequence of this intimin type to that of intimin β1. The heterogeneity observed among E. coli intimin genes and their expressed proteins not only suggests distinct phylogenetic lineages for these EHEC and EPEC subgroups but also is likely to be important for the affinity of these adhesins to their receptors. Indeed, considerable variation also has been noted in the Tir genes among EHEC and EPEC subgroups (34). In addition, host immunity to these surface-exposed proteins produced by one E. coli strain may not provide protection against intestinal colonization by E. coli strains which bear distinct intimin or Tir types (10). Furthermore, the intimin or Tir type may also play a role in determining which region of the gut is colonized (44).

Several different E. coli intimin genes have been cloned, and recombinant intimin proteins have been expressed at a high level and purified (3, 4, 12, 13, 27, 28, 47). The antisera raised against these recombinant proteins have been used to examine the degree of immunological relatedness among intimins. These antisera have been helpful in the classification of intimin types (1, 3); however, cross-reactions with other intimin types are known to occur. Zhu et al. (47, 48) reported production of monoclonal antibodies to the intimin of E. coli O45 (type β), antibodies which reacted with a homologous intimin type from rabbit diarrheagenic E. coli type 1 (RDEC-1) but not with intimin type α from E. coli O127:H6 strain E2348/69. Unfortunately, the specificities of these monoclonal antibodies with respect to other intimin types were not reported.

In this report, we characterize a monoclonal antibody specific for intimin y1 and describe the use of this antibody in specifically identifying intimin y1-producing E. coli strains.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. E. coli strains of human origin were kindly provided by M. Karmali (Health Canada Laboratory Centre for Enteric and Zoonotic Diseases, Guelph, Ontario, Canada), M. Anand (University of Guelph, Guelph, Ontario, Canada), C. Gyles (University of Guelph, Guelph, Ontario, Canada), S. M. Scotland (Central Public Health Laboratories, London, England), and M. Richter (Northern Alberta Provincial Health Laboratory, Edmonton, Alberta, Canada). E. coli strains of porcine origin were supplied by C. Gyles (University of Guelph) and J. M. Fairbrother (University of Montreal, Saint-Hyacinthe, Quebec, Canada).

PCR amplification, cloning, and nucleotide sequencing of ear genes. Oligonucleotide primers used in the study were purchased from Canadian Life Technologies (Quebec, Canada). The heterogeneity observed among E. coli intimin genes and their expressed proteins not only suggests distinct phylogenetic lineages for these EHEC and EPEC subgroups but also is likely to be important for the affinity of these adhesins to their receptors. Indeed, considerable variation also has been noted in the Tir genes among EHEC and EPEC subgroups (34). In addition, host immunity to these surface-exposed proteins produced by one E. coli strain may not provide protection against intestinal colonization by E. coli strains which bear distinct intimin or Tir types (10). Furthermore, the intimin or Tir type may also play a role in determining which region of the gut is colonized (44).

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In this report, we characterize a monoclonal antibody specific for intimin y1 and describe the use of this antibody in specifically identifying intimin y1-producing E. coli strains.

Preparation of antisera. Approximately 150 µg of each purified six-His-tagged intimin fusion protein (His-intO157, His-intO157C, and His-intO127C) was homogenized 1:1 with incomplete Freund’s adjuvant (Canadian Life Technologies). A 1-ml volume of this mixture was injected into two subcapsular and two intramuscular sites of Flemish giant hybrid rabbits (Biosciences Animal Services, University of Alberta, Edmonton, Canada). Booster doses of this vaccine preparation were given again at 4 to 6 weeks after the first vaccination, and then up to three booster vaccinations were given every 2 weeks thereafter. Two weeks after the last vaccination, the rabbits were exsanguinated and the sera

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strains were collected by centrifugation. The antisera were absorbed against heat-killed
E. coli BL21(DE3) cells by incubation of the sera with the cells for 4 hr at room

temperature, followed by incubation of the mixture at 4°C with shaking over-

night. This suspension was then spun at 12,000 × g for 10 min to remove the
bacteria, and the supernatant was collected and stored at −70°C.

Hybridoma culture supernatants were prepared by Rita Bigham, S. Druhan,
and G. Tiffin and were a kind gift from W. G. Yates of the Animal Diseases
Research Institute, Canadian Food Inspection Agency, Lethbridge, Alberta, Canada.
The immunoglobulin (Ig) type and subtype of monoclonal antibodies
derived from hybridomas were determined by using a mouse monoclonal anti-
body isotyping kit (Pierce, Rockford, Ill.) (19).

Preparation of E. coli OMPs and lysates. OMPs were prepared from E. coli
strains as described by Agin and Wolf (3), with modification. Briefly, E. coli
strains were grown overnight at 37°C in Luria-Bertani broth (LB). These cultures
were used for inoculation of 10 ml of one or more of the following media at a
ratio of 1:100, inoculum to medium: minimal essential medium (MEM), Dul-
becco’s modified Eagle’s medium (DMEM), buffered peptone water (BPW) (1%) peptone
water [Lab M balanced peptone water no. 1] supplemented with 3.5 g of
Na2HPO4·7H2O and 1.5 g of KH2PO4 (liter), M9 minimal medium (supplemented
with 44 mM NaHCO3, 0.4% glucose, and 0.1% Casamino Acids), rich broth (RB; 
LB containing 0.2% glucose), TB, and LB. The cultures were grown at 37°C with
shaking for 2 to 24 h. Bacterial concentrations were adjusted to an optical density
at 600 nm (OD600) of 1.0 for OMP preparations and an OD600 of 0.2 for simple
bacterial lysates, and a 1-ml sample of the concentrated or diluted bacterial
growth was harvested by centrifugation at 2,000 × g at 4°C for 10 min. The pellets
were resuspended in 10 mM HEPES buffer (pH 7.4) and centrifuged again. This

| Strain | Serotype | Source | Pathotype | vt PCR result | eae PCR result | Intimin type | HaeII type | Reactivity to E. coli lysate of rabbit antiserum raised against: |
|--------|----------|--------|-----------|---------------|---------------|--------------|------------|------------------------------------------------------------|
| EC226  | O86:NM   | Human  | EPEC      | −             | + α           | E            | ++         | +                                                      |
| EC990986 | O127:NM | Human  | EPEC      | −             | + α           | E            | +          | +                                                      |
| E2348/69 | O127:NM | Human  | EPEC      | −             | + α           | E            | +          | +                                                      |
| EC990987 | O142:H34| Human  | EPEC      | −             | + α           | E            | +          | +                                                      |
| H19    | O26:H11  | Human  | EHEC      | +             | + β1          | A            | ++         | −                                                      |
| EM88-3618(1) | O26:H11 | Bovine | EHEC      | +             | + β1          | A            | +++        | −                                                      |
| EA01985-91 | O26:H11 | Bovine | EHEC      | +             | + β1          | A            | ++         | −                                                      |
| EM88-4256 | O26:H11 | Bovine | EHEC      | +             | + β1          | A            | +          | −                                                      |
| 41131  | O26:H11  | Human  | EHEC      | +             | + β1          | A            | +++        | −                                                      |
| EC990983 | O26:H11 | Human  | EPEC      | −             | + β1          | A            | −          | −                                                      |
| H30    | O26:NM   | Human  | EHEC      | +             | + β1          | A            | +          | +                                                      |
| P86-4220 | O45:NM  | Porcine | VTec      | −             | − β1          | A            | +          | −                                                      |
| P87-4725 | O45:NM  | Porcine | VTec      | −             | − β1          | A            | +          | −                                                      |
| EC920142 | O69:H11 | Human  | EHEC      | +             | + β1          | A            | ++         | −                                                      |
| EM90-2768 | O111:H11 | Bovine | EHEC      | +             | + β1          | A            | +          | −                                                      |
| EM88-4108 | O111:H11| Bovine | EHEC      | +             | + β1          | A            | +          | −                                                      |
| EC200055 | O126:H2 | Human  | EPEC      | −             | − β1          | A            | −          | −                                                      |
| H18    | O128:NM  | Human  | EHEC      | +             | + β1          | A            | ++         | +                                                      |
| EC920234 | O80:NM  | Bovine | VTec      | +             | + NTa         | A            | +          | −                                                      |
| EM88-3620 | O5:NM   | Bovine | EHEC      | +             | + β1          | D            | ++         | +                                                      |
| 2340   | O5:NM    | Bovine | EHEC      | +             | + β1          | D            | +          | −                                                      |
| 5432   | O103:H2  | Human  | EHEC      | +             | + ε           | C            | ++         | −                                                      |
| 5529   | O103:H2  | Human  | EHEC      | +             | + ε           | C            | ++         | −                                                      |
| 9291   | O103:H2  | Human  | EHEC      | +             | + ε           | C            | ++         | +w                                                    |
| 35280  | O103:H2  | Human  | EHEC      | +             | + ε           | C            | ++         | +w                                                    |
| EC322  | O55:H7   | Human  | EPEC      | +             | + γ1          | B            | +++        | +                                                      |
| 33264  | O145:NM  | Human  | EPEC      | +             | + γ1          | B            | +++        | +                                                      |
| E319   | O157:H7  | Human  | EHEC      | +             | + γ1          | B            | +++        | +                                                      |
| E321   | O157:H7  | Human  | EHEC      | +             | + γ1          | B            | +          | +                                                      |
| H4420  | O157:H7  | Bovine | EHEC      | +             | + γ1          | B            | +          | +                                                      |
| LRH1   | O157:H7  | Human  | EHEC      | +             | + γ1          | B            | +          | +                                                      |
| LRH2   | O157:H7  | Human  | EHEC      | +             | + γ1          | B            | +          | +                                                      |
| LRH6   | O157:H7  | Human  | EHEC      | +             | + γ1          | B            | +          | +                                                      |
| E32511 | O157:NM  | Human  | EHEC      | +             | + γ1          | B            | +          | +                                                      |
| CL18   | O157:NM  | Human  | EHEC      | +             | + γ1          | B            | +          | +                                                      |
| 43426  | O103:H2  | Human  | EHEC      | +             | + γ2          | B            | +w         | −                                                      |
| EM87-1507 | O111   | Bovine | EHEC      | +             | + γ2          | B            | −          | −                                                      |
| 44717  | O111:H12 | Human   | EHEC      | +             | + γ2          | B            | −          | −                                                      |
| EC920018 | O111:H8 | Human  | EHEC      | +             | + γ2          | B            | −          | −                                                      |
| 52050  | O111:NM  | Human  | EHEC      | +             | + γ2          | B            | +          | −                                                      |
| K-12   | Rough    | Lab     | E. coli   | −             | − NAa         | NA           | −          | −                                                      |
| DAB    | O139:H1  | Porcine | VTec      | −             | − NA           | NA           | −          | −                                                      |
| B2F1/3 | O91:NM   | Human  | EHEC      | +             | − NA           | NA           | −          | −                                                      |

a See Gannon et al. (16).

b See Gannon et al. (16).

c See Oswald et al. (33).
d Reactivity characterized as +++ (strong), ++ (moderate), + (low), +w (weak), or −, (none).

1 NT, nontypeable.

NA, Not applicable.
washing step was repeated once more, and then the pellet was resuspended in 1 mL of 10 mM HEPES. The cells were placed on ice and disrupted with a Sonifier Cell Disruptor 200 (Branson) fitted with a microtip probe (settings, 40% duty cycle and output 6) for 40 s. For OMP preparations, debris was removed from the bacterial lysates by centrifugation at 9,000 × g at 4°C for 10 min. The supernatant was removed and centrifuged at 16,000 × g at 4°C for 30 min. The pellet obtained from this centrifugation step was resuspended in 400 µL of 10 mM HEPES (pH 7.4) containing 1% N-lauroylsarcosine (Sigma), and the mixture was incubated at room temperature with shaking for 30 min. Following this, OMPs were collected by centrifugation at 16,000 × g for 30 min at 4°C. The pellet was washed once in HEPES buffer without mixing, resuspended in 100 µL of this buffer, and stored at −20°C.

Slot and Western blot immunoassays. For slot blot immunoassays, approximatively 100 µL of each bacterial lysate was transferred onto nitrocellulose membranes by using a slot blot apparatus (Bio-Rad) according to the manufacturer’s instructions (1:200 dilution, room temperature with shaking for 30 min). Following this, OMPs were collected by centrifugation at 16,000 × g for 30 min at 4°C. The pellet was washed once in HEPES buffer without mixing, resuspended in 100 µL of this buffer, and stored at −20°C.

Table 2. Sizes of intimin-encoding DNA fragments and expressed intimin fusion proteins

| Plasmid construct | Size of fragment (bp) | Fusion protein | Protein size (kDa) | E. coli strain |
|-------------------|----------------------|---------------|-------------------|---------------|
| pET-eaeO157C      | 918                  | His-intO157C  | 40                | E319          |
| pET-eaeO157      | 2,806                | His-intO157   | 100               | E319          |
| pET-eaeO127C     | 932                  | His-intO127C  | 40                | E2348/69      |

Characterization of E. coli O157:H7 intimin y1

E. coli O157:H7 intimin y1 was expressed in E. coli BL21(DE3) and purified by Ni-NTA chromatography. The fusion protein was expressed as a 100-kDa protein which was dialyzed against PBS (pH 7.4) and stored at −80°C.

Reactivity of intimin polyclonal antisera with E. coli lysates. Rabbit antisera raised against His-intO157 reacted with lysates from all E. coli strains which produce intimin y1 [E. coli O157: H7(−), O145:NM, and O55:H7 strains]. However, among the E. coli O157:H7 strains, some strains showed low or weak reactivities (Table 1). These antisera also reacted strongly with lysates from other E. coli strains which possess other intimin types. Rabbit antisera raised against His-intO157C also reacted with all lysates from E. coli strains which produce the homologous intimin y1, except E. coli O157:H7 strain H4420 (Table 1). However, reactions were also observed for these antisera with lysates from E. coli strains which produce other intimin types. In contrast, rabbit antisera raised against His-intO127C reacted only with lysates from E. coli strains which had intimin type α (EPEC strains of serogroups O86, O127, and O142).

Expression and purification of fusion proteins. Portions of the eae gene of E. coli O157:H7 strain E319 and E. coli O127:H6 strain E2348/69 were expressed as proteins fused with the six-His tag of pET-28(+) expression vector systems. These constructs contained either the entire E. coli O157:H7 eae gene (pET-eaeO157), the 3’ ca. 918-bp segment of the E. coli O157:H7 eae gene (pET-eaeO157C), or the 3’ 933-bp segment of the E. coli O127:H6 strain E2348/69 eae gene (pET-eaeO127C) (Table 2).

The His-intO157 fusion protein was expressed as a 100-kDa protein (Fig. 1A, lane 2) from pET-eaeO157, and the His-intO157C (Fig. 1A, lane 5) and His-intO127C (Fig. 1A, lane 6) proteins were expressed as 40-kDa proteins from pET-eaeO157C and pET-eaeO127C, respectively. Although all of the His-intimin fusion proteins were of low solubility, they could be readily purified with 8 M urea (Fig. 1A, lanes 3, 6, and 9, respectively) to concentrations of 20 to 30 mg per liter of broth culture (data not shown).

Rabbit antisera raised against His-intO157 reacted with both the homologous fusion protein His-intO157 (Fig. 1B, lanes 2 and 3) and His-intO157C (Fig. 1B, lanes 5 and 6) and reacted weakly with the His-intO127C fusion protein in Western blots (Fig. 1B, lanes 8 and 9).

Reactivity of the monoclonal antibody. The mouse monoclonal antibody Inty1. C11 was of subclass IgG1 (data not shown). It reacted with His-intO157 (Fig. 1C, lane 2 and 3; Fig.
2, position H3) and His-intO157C (Fig. 1C, lanes 5 and 6; Fig. 2, position H4) intimin fusion proteins but did not react with His-intO127C in Western (Fig. 1C, lanes 8 and 9) and slot (Fig. 2) blots. While rabbit antisera raised against His-intO157 and His-intO157C cross-reacted with bacterial lysates from other EHEC and EPEC strains (Table 1), the monoclonal antibody reacted only with preparations from the intimin γ1-producing E. coli strains of serotypes O157:H7(H 7) (Fig. 2, positions A1 through B2), O145:NM (Fig. 2, position B3), and O55:H7 (Fig. 2, position B4) in slot blots.

Levels of intimin expression by E. coli O157:H7 strains in different media. Intimin γ1 expression by E. coli O157:H7 and O157:H7 strains varied and was dependent on the culture medium and bacterial strain (Fig. 3A). All strains tested produced intimin γ1 in DMEM and MEM; however, intimin γ1 production was low or not detected in LB, RB, and TB (Fig. 3A). E. coli O157 strain E319 had high intimin γ1 production relative to that of other E. coli O157 strains in all media tested except for RB and TB. E. coli strain E32511 had low intimin γ1 production in MEM and DMEM, but its production of intimin γ1 was higher in BPW and M9 medium than those of other E. coli O157:H7 strains. Among the E. coli O157:H7 strains tested, E. coli O157:H7 strain LRH6 produced the highest level of intimin γ1 in MEM and DMEM, but its production of intimin γ1 was lower in BPW and M9 medium than those of other E. coli O157:H7 strains. E. coli O157:H7 strains LRH1, LRH2, and H4420 were low-intimin γ1-producing strains. Intimin γ1 production by E. coli O157:H7 strain H4420 was detected only in MEM (Fig. 3A).

In MEM, DMEM, and M9 medium, intimin γ1 production by E. coli O157:H7 strain E319 was detected after 2 h of culture, was highest between 4 and 5 h of incubation, and did not increase after this (Fig. 3B). Intimin γ1 was detected after 3 h of incubation in BPW and LB. By contrast, very low levels of intimin γ1 production were detected at 24 h in RB and TB (Fig. 3).
tested in slot blot assays.

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Concentrations were adjusted to an OD600 of 0.2, and bacterial lysates were prepared and tested in slot blot assays. (B) *E. coli* O157:H7 strain E319 was grown in MEM, DMEM, BPW, LB, RB, and TB for 24 h at 37°C. Final cell concentrations were adjusted to an OD600 of 0.2, and bacterial lysates were prepared and tested in slot blot assays.

**DISCUSSION**

In the present study, attempts were made to determine if rabbit polyclonal antisera raised against *E. coli* O157:H7 intimin would be useful in identifying intimin γ1-producing *E. coli* strains. Antisera raised against the entire intimin polypeptide of *E. coli* O157:H7 reacted not only with His-intO157 and His-intO157C fusion proteins on Western blots but also with His-intO127C as well as bacterial lysates from a large number of other intimin-producing *E. coli* strains. Similar broad-based reactivity with intimins has been recently reported by Batchelor et al. (4) for rabbit polyclonal antisera raised against a conserved portion of intimin from *E. coli* O157:H7. This result is not surprising, given that the N-terminal and central portions of the protein appear to be reasonably well conserved among intimin types, and it is likely that many epitopes are also shared among these bacterial proteins. It is interesting to note that some of the weakest reactions with these antisera were observed for bacterial lysates from *E. coli* O157:H7 strains which would presumably produce the homologous intimin type γ1. Consequently, it is possible that the reactions observed may provide more information about the level of intimin γ1 production by the *E. coli* O157:H7 strains than about antigenic differences among the intimin types.

The rabbit polyclonal antisera raised against the C-terminal portion of intimin γ1 (His-intO157C) appeared to be more type specific than the antisera raised against the full-length His-*E. coli* O157:H7 intimin fusion protein, but these antisera also reacted with bacterial lysates from *E. coli* strains which produce other intimin types (e.g., intimins α, β1, and ε) but not with intimin γ2-producing *E. coli* strains. This suggests that there may be common epitopes among some of these other types and intimin γ1. The polyclonal rabbit antisera raised against the C-terminal portion of intimin α (His-intO127C) was much more specific and reacted only with bacterial lysates from *E. coli* strains producing intimin α (EPEC O86, O127, and O142 strains).

In contrast to the rabbit polyclonal antisera, the mouse monoclonal antibodies to intimin γ1 reacted only with the homologous intimin antigens His-intO157 and His-intO157C and not with His-intO127C in Western (Fig. 1) and slot (Fig. 2) blots. The monoclonal antibody Inty1.C11 also reacted only with OMPs and bacterial lysates from *E. coli* strains which produce intimin γ1 [serotypes O157:H7 (H−), O145:H−, and O55:H7] and did not react with OMPs or bacterial lysates from other *eae* PCR-positive *E. coli* strains. It is evident that these monoclonal antibodies are useful in the detection of intimin γ1-producing *E. coli* strains. In addition, intimin is reported to be highly immunogenic (25, 34), and it is possible that these monoclonal antibodies could be used in a blocking enzyme-linked immunosorbent assay to detect specific antibody responses to this portion of the protein.

Frankel et al. (12) reported that the C-terminal 150 amino acids and the cysteine residue at position 937 of intimin were necessary for the attachment of *E. coli* strain E2348/69 (O127:H6, intimin α) to HEp-2 cells. Recently, Gansheroff et al. (17) have also shown that rabbit polyclonal antisera raised against the C-terminal one-third of intimin γ1 block the binding of *E. coli* O157:H7 to HEp-2 cells. Therefore, it is possible that the monoclonal antibodies such as the one described in this study could prevent the attachment of intimin γ1-producing *E. coli* strains to epithelial cell lines and intestinal cells. If this is true, monoclonal antibodies such as this could be used to prevent intestinal colonization by *E. coli* in animals and humans. By way of analogy, monoclonal antibodies to the F5 (K99) adhesin have been used to prevent intestinal colonization by enterotoxigenic *E. coli* strains in calves (41). However, further study is required to determine whether monoclonal antibodies to intimin γ1 could prevent bacterial attachment to the intestine.

Recent work has shown that control of expression of the operons in the LEE pathogenicity islands of EPEC and EHEC is mediated by a complex regulatory cascade. For both EPEC and EHEC, LEE operons 1 through 3 encode components of the type III secretion apparatus, the Tir operon encodes Tir, CesT, and intimin, and operon 4 encodes the secreted Esp proteins. The LEE-encoded regulator (Ler) expressed by the first gene of operon 1 appears to positively regulate expression of genes in LEE operons 2 and 3, the Tir operon, and operon 4 (29, 43). In the EPEC strain E2348/69 (O127:H6), Ler expression has been shown to be positively regulated by at least three different factors: (i) the products of the plasmid-borne *per* locus, (ii) a quorum-sensing mechanism involving autoin-
ducer-2 (43), and (iii) a locus termed the integration host factor (14). By contrast, E. coli O157:H7 and other EHEC isolates possess neither the EPEC plasmid nor the per regulatory genes (18, 29). As with EPEC, quorum sensing involving autoinducer-2 appears to play an important role in the expression of LEE operons 1 and 2 in E. coli O157:H7 (43). In addition, the expression of E. coli O157:H7 LEE operons 3 and Tir may be activated by the alternative σ factor RopS (σ^{38}). Given these differences in LEE regulation between EPEC and E. coli O157:H7, it is not surprising that conditions affecting the expression of intimin by EPEC strain E2348/69 and E. coli O157:H7 strains should also differ. Previous studies have shown that E. coli O157:H7 adheres very poorly to epithelial cell lines such as HEp-2, INT407, and Caco-2 relative to EPEC strains (31, 40, 42). However, as with E. coli strain E2348/69 (O127:H7) (38), intimin expression by E. coli O157:H7 strain E319 is greatest during logarithmic-phase growth. In the present study, E. coli O157:H7 strain E319 intimin was first detected at 2 h after incubation in MEM and M9 medium, was greatest at 4 to 5 h after incubation, and did not appear to increase after this. The level of intimin expression by E. coli O157:H7 strains was also dependent on the culture medium and on the bacterial strain. All E. coli O157:H7(H^{-}) strains tested produced intimin in both MEM and DME medium, but intimin was detected in only a few strains grown in BPW, LB, or M9 medium.

Interestingly, little intimin expression was observed for E. coli O157 strains when they were grown in media which allowed rapid bacterial growth, e.g., RB and TB. Nishikawa et al. (31) reported that E. coli O157:H7 and other EHEC strains cultured in peptone water in the absence of mannose adhered well to HEp-2 cells, whereas bacteria grown in the presence of D-mannose or other sugars showed little adherence to the HEp-2 cells. Results of the present study would support the possibility that intimin production in E. coli O157 strains is subject to catabolite repression. It would also seem likely that intimin expression is regulated differently among the E. coli O157:H7 strains examined or that some of the strains maintained in the laboratory may have lost certain regulatory elements. While it may be argued that some of the differences in intimin expression observed among E. coli O157:H7 strains may be artifacts that represent differences in reactivity to the monoclonal antibody, results with polyclonal antibody to the full-length intimin also suggest differences in the levels of intimin expression among these E. coli O157:H7 strains (see Table 1). Recently, Ogierman et al. (32) have reported the isolation of a strain of E. coli O157:H7 from a human patient which lacks attaching and effacing activity and produces very low levels of intimin. These workers demonstrated that this low-level intimin production is attributable to a mutation in the ler gene which results in an amino acid substitution in this important regulatory protein. It is possible that some of the E. coli O157:H7 strains producing low levels of intimin that were examined in this study, e.g., E. coli O157:H7 strain H4420, may also have defects in this or other regulatory genes. Further study is required to characterize the genetic features associated with differences in intimin expression among E. coli O157:H7 strains. This information will be helpful in understanding the pathogenesis of infection caused by this bacterial pathogen and the conditions which would be helpful for the immunological detection of intimin γ1-producing E. coli strains.

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