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Effect of Enteropathogenic *Escherichia coli* on Adherent Properties of Chinese Hamster Ovary Cells

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Enteropathogenic *Escherichia coli* (EPEC) O111:H2, O119:H6, or O142:H6 caused rapid detachment of Chinese hamster ovary (CHO) cell monolayers within 2 to 4 h of cocultivation. CHO cell detachment was not promoted by nonenteropathogenic *E. coli* (O125:H4, O126:H27, O157:H7, and O26:H11) and could not be attributed to EPEC production of enterohemolysin or Shiga-like toxins. In contrast, EPEC strains did not promote rapid detachment of Lec1, Lec2, or Lec8 CHO cell monolayers. These CHO cell Lec mutants all express abbreviated glycan sequences on membrane glycoproteins and glycolipids. Although EPEC strains failed to alter the adherent properties of Lec2 cells lacking only terminal sialic acid groups, EPEC adherence to the Lec2 mutant was indistinguishable from that observed with wild-type CHO cells. There was also no significant difference in EPEC-induced actin accumulation or invasion of Lec2 cells. In contrast, EPEC localized adherence to Lec1 and Lec8 mutants, lacking sialyllactosamine (Lec1) or sialic acid and galactose (Lec8) sequences, was reduced by 84 and 93%, respectively. Our results suggest that lactosamine sequences [βGal(1-4 or 1-3)βGlcNAc] not containing sialic acid are sufficient for EPEC adherence, actin accumulation, and invasion of CHO cells. Sialic acid groups, however, may be necessary for EPEC-mediated CHO cell detachment.

Escherichia coli comprises a heterogeneous group of microorganisms with wide-ranging potential for interacting with their hosts. There are three broad categories into which *E. coli* can be subdivided: (i) nonpathogenic *E. coli* which constitute the normal flora of the host, (ii) those organisms, like the uropathogenic *E. coli*, which are opportunistic pathogens, and (iii) those organisms which are true pathogens. The diarrheagenic *E. coli* organisms are true pathogens and can be further subdivided into at least five, and perhaps six, groups based on defined clinical symptoms and virulence mechanisms. These groups include enteroinvasive *E. coli*, enterotoxigenic *E. coli*, enterohemorrhagic *E. coli*, enteroaggregative *E. coli*, and enteropathogenic *E. coli* (EPEC). Recent studies suggest that a sixth group, diffusely adherent *E. coli*, may also be included in this list (11).

Production of clinical symptoms by EPEC is a complex, multistep process initiated by bacterial attachment to intestinal epithelial cells and involves plasmid and chromosomal gene products (11, 12). The EAF (EPEC adherence factor) plasmid codes for the ability of EPEC to attach to HEp-2 cells (2) in a typical localized adherence (LA) pattern (8, 38). Knutton et al. demonstrated that the EAF plasmid was involved in the initial, nonintimate adherence of EPEC to epithelial cells (26). More recently, the EAF plasmid was found to code for bundle-forming pili which cause aggregation of EPEC grown in tissue culture medium and are responsible for the LA phenotype (17, 18, 40).

EPEC organisms also possess chromosomal *eae* (EPEC attaching and effacing) genes (11, 22, 24, 35) which, following the initial EAF-mediated attachment of the bacteria to epithelial cells, cause attaching and effacing lesions in vivo (32) and in vitro (16, 28). These lesions are characterized by intimate association of the bacteria with the epithelial cell surface, resulting in localized destruction of the microvilli and accumulation of actin (27) and other cytoskeletal components (15, 30) at the sites of bacterial attachment. The presence of this accumulated actin forms the basis of the fluorescent-actin stain (FAS) test, which is used to identify EPEC (27). Jerse and Kaper demonstrated that the formation of attaching and effacing lesions is regulated in *trans* by an EAF plasmid gene(s) (22).

Although HEp-2 or HeLa cells are commonly used to study EPEC LA and invasion of eukaryotic cells, we have found that Chinese hamster ovary (CHO) cells represent an alternative in vitro model system for studying these virulence mechanisms. Moreover, in this article, we show that EPEC causes a highly reproducible and distinctive loss of CHO cell monolayers in addition to attachment and invasion. CHO cell (Lec) mutants expressing altered surface oligosaccharide sequences (9, 10, 39) also appear to be useful for investigating the requirement

TABLE 1. Characteristics of *E. coli* strains

E. coli strain	Enterohemolysin[a]	SLT[b]	Rapid loss of CHO cell monolayers	FAS test		*eae* gene[c]	EAF plasmid[d]
				CHO	HEp-2		
O111:H2	−	−	+	+	+	+	+
O119:H6	−	−	+	+	+	+	+
O125:H4	−	−	−	−	−	−	ND[e]
O126:H27	−	−	−	−	−	−	ND
O142:H6	−	−	+	+	+	+	+
O157:H7	+	SLT-II/SLT-I	−	ND	ND	+	−
O26:H11	+	SLT-I	−	+	ND	+	−

[a] Determined by the method of Beutin et al. (5).
[b] Determined by the method of Armstrong et al. (1). Shiga-like toxins (SLT) 50% cytotoxic dose = 0.4 pg/ml.
[c] Determined by the method of Louie et al. (29).
[d] Determined by the method of Mass and Jerse et al. (23, 31).
[e] ND, no data.

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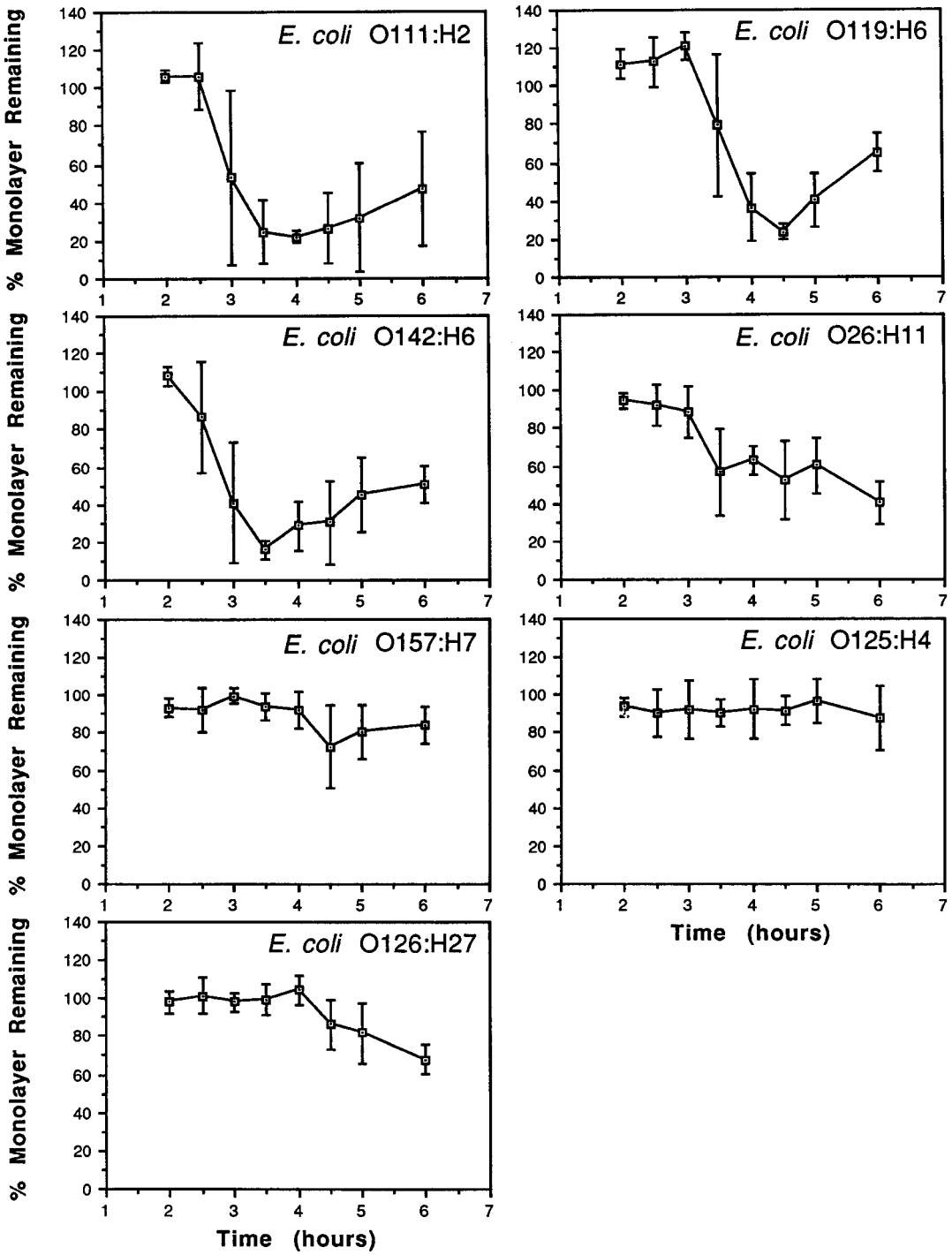

FIG. 1. Effect of bacteria on adherent properties of wild-type CHO cells. After incubating with bacteria for various times, CHO cells remaining attached to the tissue culture wells were fixed and stained with Giemsa stain. Stained cells were then lysed with 2% SDS, and the A_{620}s of these solutions were used to calculate the percent monolayer remaining relative to uninfected monolayers.

for sialyl and asialo-glycans by EPEC for LA, invasion, and detachment of CHO cell monolayers.

MATERIALS AND METHODS

Bacteria. The relevant characteristics of all bacteria used in this study are listed in Table 1. Enterohemorrhagic *E. coli* O157:H7 was provided by L. Linarez (Provincial Laboratory of Northern Alberta, Edmonton, Canada). *E. coli* O26:H11 (strain H19) was obtained from S. M. Scotland (Division of Enteric Pathogens, Central Public Health Laboratory, London, United Kingdom). All bacteria were grown aerobically at 37°C in tryptic soy broth (TSB) (Difco, Detroit, Mich.).

Tissue culture. CHO-K1 (CCL 61) cells and CHO cell mutants Lec1 (CRL 1735), Lec2 (CRL 1736), and Lec8 (CRL 1737) were obtained from the American Type Culture Collection (Rockville, Md.). The phenotype of the Lec mutants was confirmed by a previously published procedure (20). HEp-2 cells were provided by L. Chui (Provincial Laboratory of Northern Alberta). All tissue culture cells were grown at 37°C in an atmosphere containing 5% CO_2 and 95%

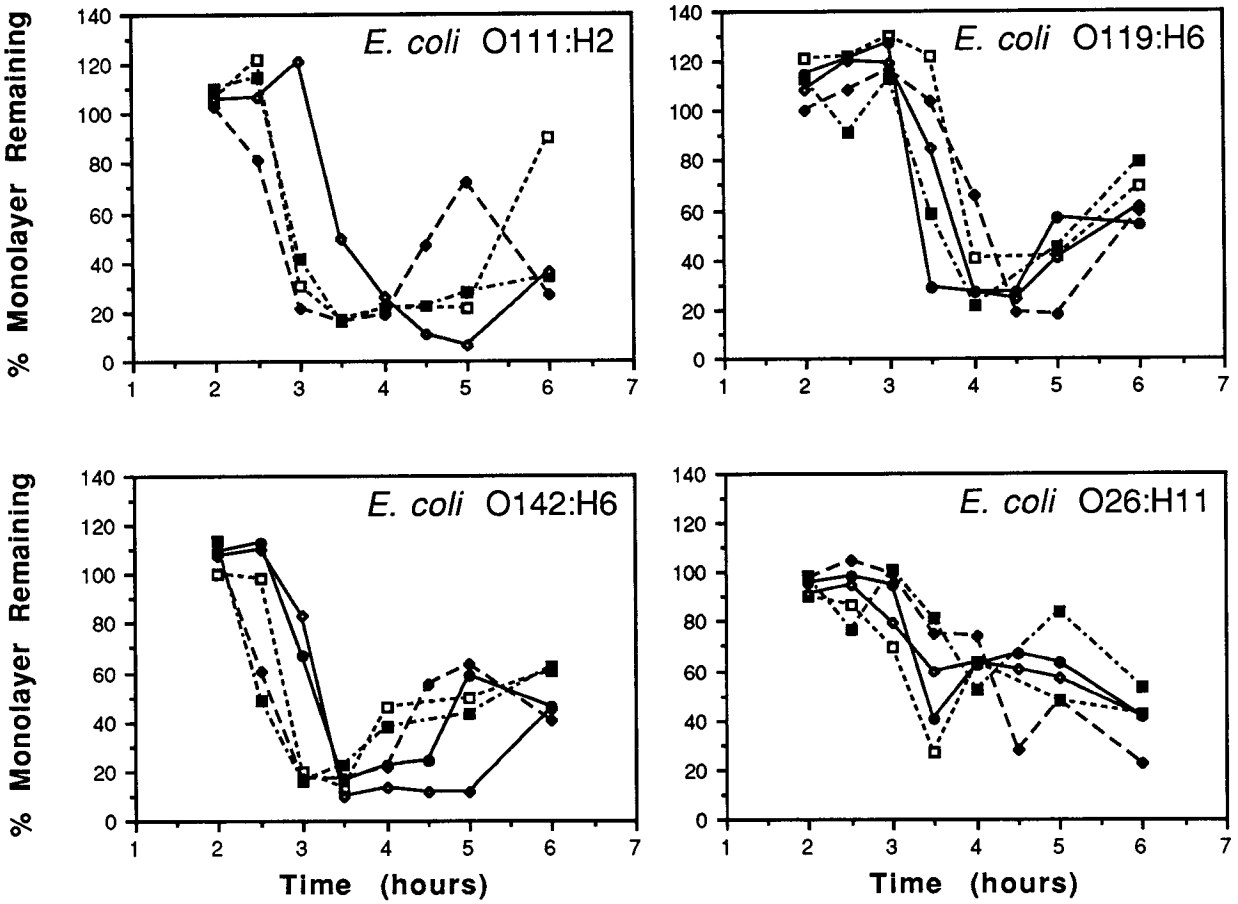

FIG. 2. Rate of CHO cell monolayer loss caused by EPEC strains (O111:H2, O119:H6, and O142:H6) and non-EPEC O26:H11. Individual lines in each of the panels illustrate the CHO cell response for independent experiments for each bacterial strain: trial 1 (– –□– –), trial 2 (– · –■– · –), trial 3 (—◇—), trial 4 (– –◆– –), and trial 5 (—●—). Experimental details are given in the legend to Figure 1.

air in medium supplemented with 10% fetal bovine serum (FBS). All reagents for tissue culture were obtained from Gibco (Burlington, Ontario, Canada). HEp-2 cells were grown in minimal essential medium with Earle's salts (MEM). Unless indicated otherwise, CHO cells were grown in F-12 nutrient mixture (Ham), and Lec1, Lec2, and Lec8 cells were grown in MEM (α medium) as recommended by the American Type Culture Collection. For some experiments, however, Lec2 cells were cultured separately in F-12 nutrient mixture (Ham) for a minimum of 10 passages prior to use. Confluent monolayers were disrupted with a solution consisting of 0.25% (vol/vol) trypsin in FC buffer (0.14 M NaCl, 5.0 mM KCl, 20.0 mM Tris HCl, 5.0 mM Tris base, 0.5 mM EDTA [pH 7.2]). The cells were resuspended in the appropriate medium, and 1.25×10^5 cells were added to each well of the 24-well tissue culture plates. The plates were incubated at 37°C for 24 to 72 h, depending on the experiment and cell line. Subconfluent monolayers were used for the FAS test and LA assay, while confluent monolayers were used for all other experiments.

Bacterial replication in tissue culture medium. Approximately 2.0×10^6 (5 to 15 μl) TSB-grown, log-phase organisms (determined by measuring culture turbidity at 600 nm) were added to 0.5 ml of FBS-supplemented F-12 nutrient mixture (Ham) per well in 24-well tissue culture plates in the absence of CHO cells. The number of viable organisms added was confirmed by performing plate counts from serial dilutions of the inocula. After incubation for 3 h at 37°C in a CO_2 incubator, aliquots were removed from the wells and serially diluted in order to determine the number of viable organisms. Individual experiments were done in duplicate and repeated twice.

EPEC-induced detachment of CHO cells. Tissue culture plates were prepared as described previously using CHO, Lec1, Lec2, and Lec8 cells. After the monolayers became confluent, the tissue culture medium was replaced with 0.5 ml of fresh medium containing 10% FBS and 0.5% D-mannose (8, 27), and approximately 2.0×10^6 TSB-grown, log-phase organisms were added to each well. Experiments were performed in the presence of D-mannose to prevent type 1 pilus-mediated bacterial attachment. Following incubation at 37°C, the monolayers were washed three times with phosphate-buffered saline (PBS) to remove nonadherent CHO cells. CHO cells remaining in each well were fixed for 10 min with methanol and then stained with Giemsa stain for 30 min. The monolayers were then washed three times with water to remove excess Giemsa stain, and the stained cells were lysed with 2% sodium dodecyl sulfate (SDS). Solutions of lysed cells were transferred to 96-well microtiter plates, and the absorbance of the contents from each well was recorded using a Titertek Multiskan MC microtiter plate reader set at 620 nm. The percentage of monolayer remaining was calculated as follows: (A_{620} of inoculated well/A_{620} of uninoculated well) × 100. At least four independent experiments were performed for each strain.

FAS test. The FAS test (27) was done as follows for all bacteria except *E. coli* O26:H11 and O157:H7. Briefly, subconfluent HEp-2 cell monolayers were grown on 12-mm-diameter glass coverslips in 24-well plates as described above. Five microliters of an overnight bacterial broth culture was then added to each well in the presence of HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-buffered (pH 7.2) MEM with 2% FBS and 0.5% D-mannose. After incubation at 37°C in a CO_2 incubator for 3 h, the coverslips were washed 3 times with PBS. The cells were then fixed for 20 min in 3% formaldehyde and then washed another three times with PBS. Next, the formaldehyde-fixed HEp-2 cells were treated with 0.1% Triton X-100 in PBS for 5 min, and the permeabilized monolayers were washed another three times with PBS and then treated with a 5-μg/ml solution of fluorescein isothiocyanate-conjugated phalloidin (phalloidin-FITC; Sigma Chemical Company, St. Louis, Mo.) in PBS for 20 min to stain actin. After being washed three times with PBS to remove excess phalloidin-FITC, the coverslips were mounted in 80% glycerol–PBS and viewed with a fluorescence microscope fitted with a 340- to 380-nm excitation filter.

When performing the FAS test on EPEC with CHO, Lec1, Lec2, or Lec8 cells, it was necessary to reduce the cocultivation time from 3 to 2 h prior to staining the monolayers with phalloidin-FITC. Essentially the same procedure was followed for *E. coli* O26:H11 and O157:H7 except that the bacteria were cocultivated with CHO cells for a total of 6 h. At 3 h of incubation with O26:H11 and O157:H7 the CHO cell monolayers were washed and fresh medium was added to prevent bacterial overgrowth. F-12 nutrient mixture (Ham) or MEM (α medium) supplemented with 10% FBS and 0.5% D-mannose was used for cocultivation of EPEC or of O26:H11 or O157:H7 with CHO and Lec cells, respectively. The monolayers were then stained with phalloidin-FITC and observed with a fluorescence microscope as described above.

LA assay. Subconfluent monolayers of CHO, Lec1, Lec2, and Lec8 cells were prepared on 12-mm-diameter glass coverslips in 24-well plates as described previously. Following the addition of fresh growth medium containing 10% FBS and 0.5% mannose, approximately 10^9 bacteria were added to each well and the plates were incubated for 1.5 h at 37°C. The monolayers were washed five times with PBS to remove nonadherent bacteria and then fixed and stained with Giemsa stain as described earlier. Excess stain was removed by washing the coverslips three times with water. The coverslips were air dried and mounted on glass slides for viewing using the 100× oil immersion objective of a Reichert light microscope. Between 100 and 125 randomly chosen tissue culture cells were observed, and only those having five or more bacteria attached as a cluster were scored as positive for LA (40). Each experiment was repeated three times.

EPEC binding and invasion assays. Invasion assays were performed using confluent monolayers of CHO and Lec2 cells which were prepared as described earlier. The tissue culture medium was replaced with 0.5 ml of fresh medium containing 10% FBS and 0.5% D-mannose, and approximately 2.0×10^6 TSB-grown, log-phase bacteria were added to each well. Following incubation at 37°C for 1.5 h, the monolayers were washed three times with PBS and then incubated with gentamicin (100 μg/ml in tissue culture medium) for 1 h at 37°C in order to kill extracellular bacteria (14). After three washes with PBS, the tissue culture cells were lysed by treating them with 200 μl of 1% (vol/vol) Triton X-100 in PBS for 5 min at 37°C. The lysed suspensions were then diluted to 1 ml with TSB, and the number of intracellular bacteria was determined from serial dilutions of this mixture.

For bacterial binding assays, the above procedure was followed except that incubation of the monolayers with gentamicin followed by three washes with PBS was omitted. Both assays were repeated four or five times.

Statistical analysis. Statistical analyses (nonparametric Wilcoxon test or *t* test) were performed using SYSTAT (Evanston, Ill.) software on a Macintosh Plus computer.

RESULTS

Detachment of CHO and Lec cell monolayers. Of all the bacteria tested in this assay, only the EPEC isolates (O111:H2, O119:H6, and O142:H6) caused detachment of CHO cell monolayers within 2 to 4 h of cocultivation (Fig. 1). The large error bars in this figure were the result of interexperimental variation in the time (±30 min) of onset of the effect (Fig. 2). However, regardless of the time of onset of monolayer detachment, the rate at which detachment occurred was highly reproducible and always greater than that observed in CHO cells infected with non-EPEC.

E. coli O125:H4 and O157:H7 caused essentially no detachment of CHO cell monolayers over the 6-h incubation period, whereas *E. coli* O26:H11 and O126:H27 caused gradual detachment of the monolayers after 3 to 4 h of incubation. However, the changes in the adherence of CHO cells caused by *E. coli* O126:H27 and O26:H11 were not characteristic of the rapid changes caused by EPEC.

Lec1 CHO cell mutants lack an *N*-acetylglucosaminyltransferase (GlcNAc-T1) required for transferring *N*-acetylglucosamine to α-1,3-linked mannose exclusively in asparagine-linked (N-linked) glycans on cell surface glycoproteins (39). Consequently, Lec1 cells lack the *N*-acetylglucosamine termini which are required for completing the biosynthesis of sialo [αNeu5NAc(2-6,3)βgal(1-4,3)βGlcNAc] and asialo [βgal(1-4,3)βGlcNAc] lactosamine sequences. Lec2 cells are unable to translocate CMP-sialic acid across Golgi vesicle membranes and are deficient in incorporating sialic acid into glycan sequences on both glycoproteins and glycolipids (10). Lec8 mutants are unable to translocate UDP-galactose into the lumen of the Golgi apparatus and are deficient in galactose incorporation into glycans on glycoproteins and glycolipids (9). Therefore, Lec8 cells lack terminal sialic acid in addition to galactose (sialic acid acceptors) on glycoprotein and glycolipid oligosaccharide sequences. The rate of EPEC-mediated detachment of Lec1, Lec2, or Lec8 cell monolayers was not as rapid as that observed with wild-type CHO cells (Fig. 3).

In order to ensure that the absence of rapid monolayer detachment observed using Lec cell lines was not due to the different media used to culture CHO and Lec cells (F-12 nu-

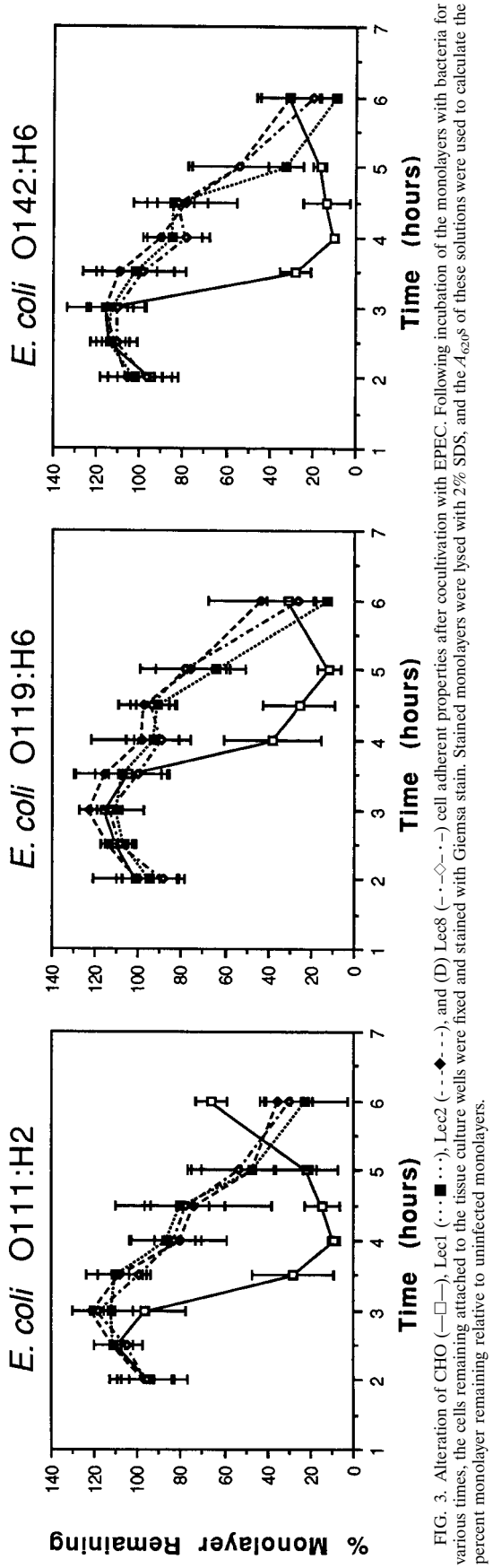

FIG. 3. Alteration of CHO (—□—), Lec1 (···■···), Lec2 (- - -◆- - -), and (D) Lec8 (–·–◇–·–) cell adherent properties after cocultivation with EPEC. Following incubation of the monolayers with bacteria for various times, the cells remaining attached to the tissue culture wells were fixed and stained with Giemsa stain. Stained monolayers were lysed with 2% SDS, and the A_{620}s of these solutions were used to calculate the percent monolayer remaining relative to uninfected monolayers.

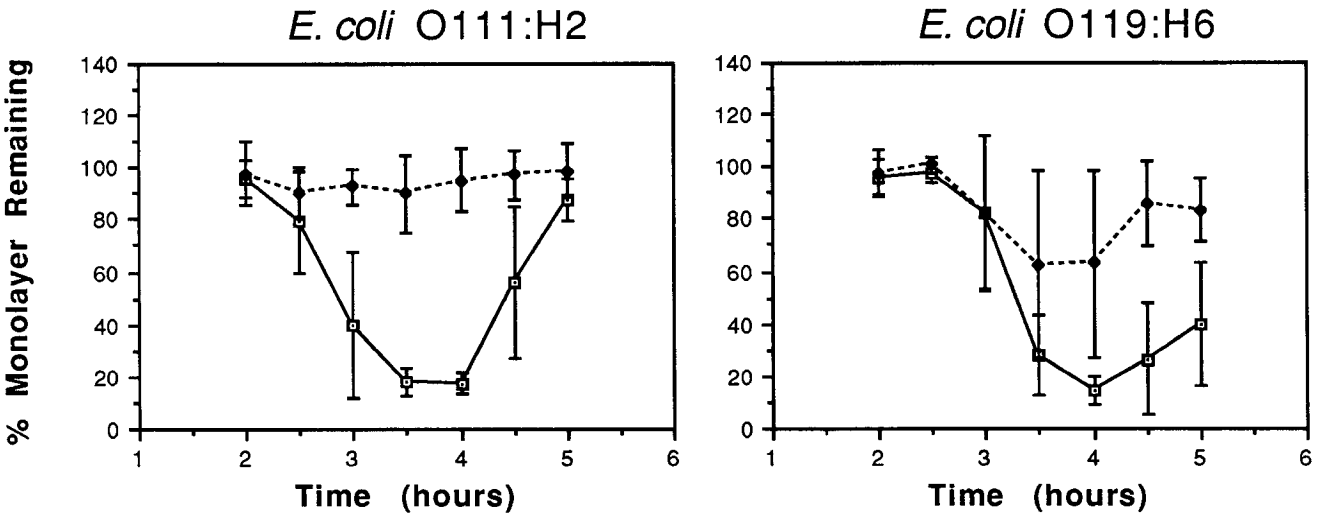

FIG. 4. EPEC-induced detachment of CHO (—⊡—) and Lec2 (– – –◆– – –) cells cultured in F-12 nutrient mixture (Ham). Lec2 cells were cultured in F-12 nutrient mixture for a minimum of 10 passages before use. Monolayer detachment assays were performed as described in Materials and Methods.

trient mixture [Ham] and MEM [α medium], respectively), experiments were repeated using Lec2 cells cultured in F-12 nutrient mixture (Ham). EPEC were unable to induce rapid loss of Lec2 cell monolayers grown in the same medium as wild-type CHO cells (Fig. 4). Furthermore, there was no significant difference in EPEC attachment ($P > 0.593$) to CHO and Lec2 cells cultured in this medium.

Cytotoxins produced by *E. coli* strains. Enterohemolysin or Shiga-like toxins (SLTs) were not responsible for loss of the CHO cell monolayers since neither O157:H7 nor O26:H11 *E. coli*, both of which produce enterohemolysin and SLTs (Table 1), caused a rapid loss of the monolayers similar to that caused by EPEC. Furthermore, we consider it unlikely that the alteration of CHO cell adherent properties was due to the production of cytolethal distending toxin (CDT) (25) by EPEC since cytotoxic effects caused by CDT require 96 to 120 h for observation. In contrast, loss of CHO cell monolayers in our study occurred after 2 h of incubation.

Bacterial replication in tissue culture medium. To determine if the loss of CHO cell monolayers was due to more rapid replication of the EPEC, the growth of bacteria in tissue culture medium was determined. However, no significant differences ($P > 0.068$) were detected in the growth of any of the strains in tissue culture medium (data not shown). Therefore, the loss of the monolayer was not due to more rapid growth of the EPEC.

Adherence characteristics of EPEC. Only EPEC, as identified by both their possession of the EAF plasmid and their positive reaction in the FAS test (Table 1), caused the rapid detachment of CHO cell monolayers. These isolates were found to possess both *eae* gene and EAF plasmid sequences, which may also be used to identify EPEC. Although *E. coli* O26:H11 and O157:H7 also possess *eae* gene sequences, EPEC EAF sequences were not detected in these serotypes. *E. coli* O26:H11 also caused actin accumulation at sites of bacterial attachment to CHO cells. However, parallel experiments in which adherent bacteria were stained with Giemsa stain demonstrated that very few *E. coli* O26:H11 adhered to CHO cells as compared with the EPEC isolates. It is not known, therefore, if the inability of *E. coli* O26:H11 to cause a rapid detachment of CHO cell monolayers was the result of lower numbers of adherent bacteria or if the rapid loss of the monolayers is an effect unique to EPEC. A positive FAS result was not observed for the *E. coli* O157:H7 isolate because these bacteria completely failed to adhere to CHO cells.

EPEC LA in Lec cells. There was no significant difference ($P = 0.314$) in LA of EPEC strains to Lec2 or wild-type CHO cells (Table 2). This indicated that, although glycan sequences terminating in sialic acid were important for mediating changes in CHO cell adherent properties, LA does not require sialylated glycans. However, significantly fewer Lec1 ($P = 0.008$) and Lec8 ($P = 0.008$) cells had localized adherent bacteria. These results suggest that lactosamine sequences are required for expression of LA in CHO cells. Moreover, Lec1 mutants, which express altered N-linked glycan but not O-linked glycan or glycolipid sequences, bound significantly more ($P = 0.011$) EPEC than did Lec8 cells, which have defects in both glycoprotein and glycolipid sequences. Therefore, lactosamine sequences in both glycoproteins and glycolipids appear to play a role in LA of EPEC to CHO cells.

EPEC interaction with CHO and Lec2 cells. Actin accumulation caused by *E. coli* O119:H6 was similar in CHO cells and Lec2 cells (Fig. 5). Comparable results were seen with EPEC O111:H2 and O142:H6 (data not shown). These results suggest that sialic acid residues in glycoproteins or glycolipids on CHO cells are not required for EPEC-induced actin accumulation in these cells. EPEC-induced actin accumulation in Lec1 or Lec8 cells was not observed, probably resulting from the poor attachment of the bacteria to these cells. Further, there was no significant difference in the ability of *E. coli* O111:H2 ($P = 0.715$), O119:H6 ($P = 0.225$), or O142:H6 ($P = 0.144$) to invade CHO or Lec2 cells (Fig. 6). Therefore, sialic acid res-

TABLE 2. Percent CHO and Lec cell mutants with localized adherent EPEC

E. coli strain	% Cells with localized EPEC[a]			
	CHO	Lec1	Lec2	Lec8
O111:H2	86.2 ± 3.6	15.6 ± 11.7	91.5 ± 4.1	9.6 ± 5.6
O119:H6	89.1 ± 4.8	17.7 ± 12.4	88.4 ± 2.7	4.4 ± 1.9
O142:H6	88.7 ± 1.8	15.5 ± 10.9	89.1 ± 4.6	5.7 ± 3.5

[a] Mean ± standard deviation of three independent experiments.

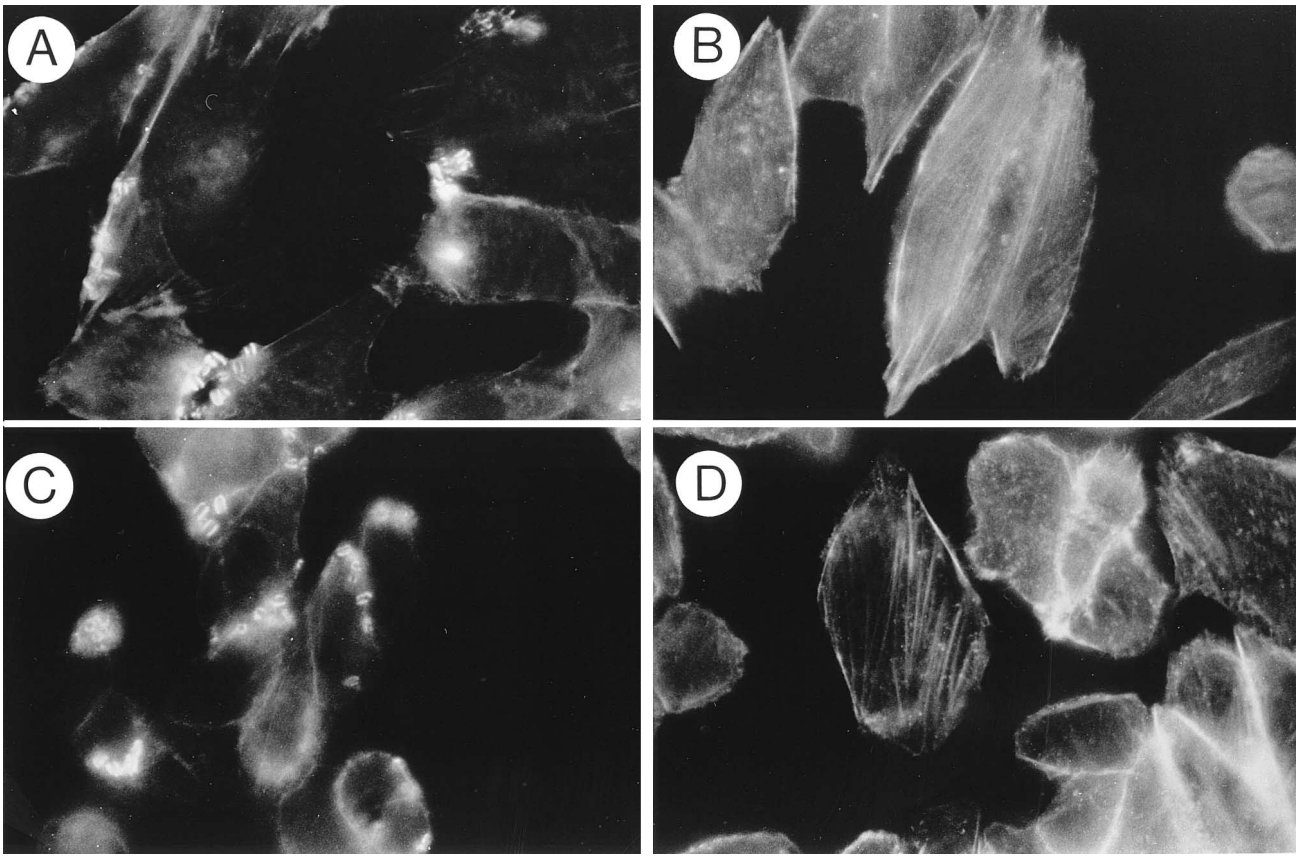

FIG. 5. *E. coli* O119:H6-induced actin accumulation in CHO (A), Lec1 (B), Lec2 (C), and Lec8 (D) cells. After *E. coli* O119:H6 was incubated with tissue culture monolayers in the presence of 0.5% D-mannose for 2 h, the cells were permeabilized with 0.1% Triton X-100 and then stained with FITC-phalloidin. Monolayers were viewed with a fluorescence microscope in order to detect actin accumulation in tissue culture cells.

idues also do not appear to play a major role in EPEC invasion of CHO cells.

DISCUSSION

According to the proposed model for EPEC pathogenesis (11, 12), EPEC attachment to epithelial cells is a multistep process. Initially, EPEC bacteria adhere to epithelial cells in a characteristic, LA pattern. Subsequently, intimate interaction occurs resulting in cytoskeletal rearrangement within the enterocyte. This is followed by EPEC invasion of the eukaryotic cell. The last processes are accompanied by an increase in intracellular calcium levels (4, 13) and protein phosphorylation (3, 30, 35). Although the pharmacological effects of EPEC interaction with eukaryotic cells are becoming better understood, the biochemical nature of the receptors involved remains to be determined. Moreover, it is still uncertain whether attachment and invasion of eukaryotic cells are sufficient to cause all the clinical symptoms associated with EPEC infections.

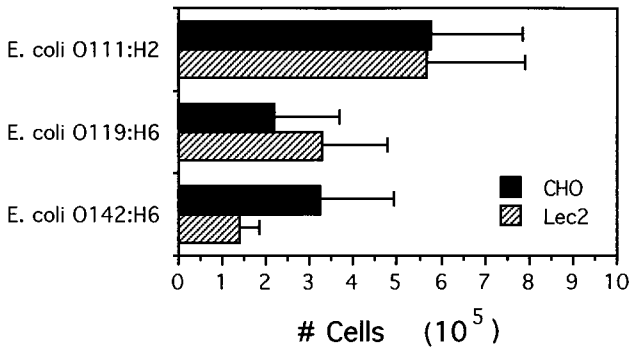

FIG. 6. EPEC invasion of CHO and Lec2 cells. Following incubation of *E. coli* O111:H2, O119:H6, and O142:H6 with CHO or Lec2 cells for 1.5 h in the presence of 0.5% D-mannose, the monolayers were incubated with gentamicin for 1 h and then lysed with 1% Triton X-100. Lysed cell suspensions were diluted in TSB, and serial dilutions were performed to determine the number of invasive bacteria.

Results published previously by Rafiee et al. demonstrated that AF/R1 pili of RDEC-1, an enteropathogenic *E. coli* strain of rabbits, bind to sialated glycoprotein complexes in rabbit ileal microvillus membranes (34). Lec1 cells, which were used in our experiments, lack *N*-acetylglucosaminyltransferase (GlcNAc-T1) activity, which results in the expression of oligosaccharide structures terminating in mannose on the surface of these cells (39). O-linked oligosaccharides and glycolipids in this mutant are the same as those present in wild-type CHO cells. In our experiments, the significant reduction in the number of Lec1 cells having locally adherent EPEC suggested that sialyllactosamine in glycoprotein sequences on CHO cells are important for the initial bundle-forming pilus-mediated attachment of EPEC (18) (Table 2). However, no significant difference was observed between EPEC LA to wild-type CHO cells or Lec2 CHO cell mutants, which are deficient only in sialic

acid incorporation into glycoproteins and glycolipids (10). Therefore, in contrast to the findings of Rafiee et al. (34) using rabbit EPEC, our data suggest that sialic acid groups are not required for LA of human EPEC in CHO cells.

The recognition of asialo-glycans on CHO cells by EPEC in our study is consistent with results published by Jagnnatha et al. (21) indicating that EPEC bound to asialo-glycolipids immobilized on thin-layer chromatography plates. Lec8 cells, which are defective in both glycoprotein and glycolipid biosynthesis (9), were more impaired than Lec1 (defective only in N-linked glycans on glycoproteins) cells in their ability to bind EPEC (Table 2). This suggested that oligosaccharide sequences in glycolipids also play a role in EPEC attachment.

The role of sialated sequences in signal transduction processes following intimate attachment by EPEC was examined using CHO and Lec2 cell mutants. There was no observable difference in actin accumulation caused by any of the three EPEC strains (O111:H2, O119:H6, and O142:H6) in either CHO or Lec2 cells. Also, there were no significant differences in the numbers of EPEC which invaded CHO or Lec2 cells. Further investigation is required to determine whether mechanisms responsible for CHO monolayer detachment are distinct from signal transduction processes associated with EPEC attachment and invasion (i.e., intracellular calcium mobilization and protein phosphorylation).

When grown at 37°C pathogenic *Yersinia* spp. express two plasmid-encoded proteins, YopE and YopH, that are cytotoxic for HEp-2 cells and mouse macrophages (6, 7, 19, 33, 37). Further, intimate attachment of yersiniae to the host cell membrane is apparently essential for functional expression of YopE and YopH cytotoxicity (33, 36). The activity of these *Yersinia* virulence factors can be monitored by monolayer detachment assays similar to the one described in this article.

Although we do not, as yet, have any data, we are tempted to speculate that EPEC, like yersiniae, may express other virulence factors in addition to those involved in attachment and invasion of eukaryotic cells. These do not appear to be enterohemolysin or Shiga-like toxins, as none of these toxins were detected in the EPEC strains used in our experiments. Further, the EPEC factor(s) responsible for CHO cell detachment may not be cytotoxic since the effect appeared to be partially reversible within the time frame of our experiments (Fig. 1 and 2).

Our results demonstrated that EPEC strains failed to cause rapid detachment of Lec2 cell monolayers similar to that seen with wild-type CHO cells. EPEC attachment to and invasion of CHO cells do not appear to be directly responsible for CHO cell detachment since these processes were normal in Lec2 mutants. Our laboratory is currently investigating the biochemical basis for EPEC-mediated detachment of CHO cell monolayers with the objective of revealing additional virulence mechanisms in these complex intestinal pathogens. The CHO cell detachment assay will be useful in obtaining EPEC mutants that are defective in the expression of this phenotype.

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