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Cloning and Expression of an Adhesin (AIDA-I) Involved in Diffuse Adherence of Enteropathogenic *Escherichia coli*

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The adherence of enteropathogenic *Escherichia coli* (EPEC) to the small bowel mucosa is an important step in the pathogenesis of diarrheal diseases. It has been shown that many EPEC strains adhere to HEp-2 and especially HeLa cells in characteristic patterns termed localized adherence (LA) and diffuse adherence (DA). A plasmid-derived DNA fragment encoding a factor specific for LA hybridized only to EPEC strains expressing LA, which demonstrated that LA and DA are mediated by two genetically distinct adhesins. EPEC strain 2787 (O127:H27), isolated from a case of infantile diarrhea, exhibited three major properties: (i) it showed DA to HeLa cells, (ii) it carried two large (ca. 100-kilobase [kb]) plasmids and one small plasmid of about 3 kb, and (iii) no fimbriae could be detected by electron microscopy in organisms grown on agar plates or in liquid cultures. Whole isolated plasmid DNA was partially digested with *Eco*RI and cloned into the vector pBR322. One recombinant clone (pIB6) was found to exhibit the same DA pattern on HeLa cells as did the parent strain. This clone contained an 11-kb DNA fragment derived from the largest of the three plasmids, as shown by Southern hybridization. By deletion analysis, a 6.0-kb DNA fragment was shown to be sufficient for expression of the DA phenotype. This insert encoded the production of a 100,000-dalton protein mediating adhesion to HeLa cells.

Enteropathogenic *Escherichia coli* (EPEC) strains were the first *E. coli* strains recognized as important pathogens in diarrheal diseases (10). Today, EPEC strains persist as a major bacterial cause of neonatal and infantile gastroenteritis throughout the world. Especially in developing countries, these strains contribute significantly to the extremely high mortality rates observed in certain areas among very young children. However, despite the recognized clinical and epidemiological impact of EPEC-caused diarrheal diseases, the virulence mechanisms used by these intestinal pathogens are only now beginning to be unraveled.

Originally, EPEC strains were classified by serotype (10), with O26, O55, O86, O111, O125, O126, O127, and O128 being the most common serotypes observed. The term EPEC is now more often used to denote *E. coli* strains that cause diarrhea but do not produce heat-stable or heat-labile enterotoxins and are not invasive according to the Serény test (12). Some strains, however, have been found to produce cytotoxins distinct from heat-stable or heat-labile toxin, such as Shiga-like toxin (19), which might also act as enterotoxins. As indicated by histopathological studies, adherence of EPEC to the intestinal mucosa seems to be of prime importance for the pathogenesis of EPEC-caused diarrhea (8, 9, 20). Attachment parallels the local effacement of microvilli of brush border membranes. These observations, together with the demonstrated importance of adhesins for the virulence of other intestinal and extraintestinal pathogenic *E. coli* strains, prompted investigations into the role of adhesins in EPEC-caused diarrheal diseases.

By using the attachment of EPEC strains to HeLa and HEp-2 cells in tissue culture as model systems, two distinct patterns of adherence were observed: localized adherence (LA), in which bacteria attach to certain areas of the cell surface and form microcolonies, and diffuse adherence (DA), in which bacteria adhere evenly to the whole cell (18, 22). An additional "aggregative pattern" of adherence was recently described by Vial et al. (23). These different attachment patterns imply recognition of different receptors on the epithelial cell surface. A large (ca. 100-kilobase [kb]) plasmid (pMAR2) harbored by an O127:H6 EPEC strain has been shown by Baldini et al. (1) to code for the LA phenotype. Two distinct regions in the plasmid spanning about 20 kb have been found to operate in *trans* for expression of the EPEC adherence factor (EAF) (17). Different gene products have been implicated as facilitating localized adherence to HEp-2 cells. Recently, Scaletsky et al. characterized two proteins of 29 and 32 kilodaltons (kDa) which were detected in outer membrane preparations of the bacterial cell wall (21). Earlier, a 94-kDa protein was suggested by Levine et al. (13) to mediate the LA phenotype. The EAF-encoding DNA fragment hybridized with DNA from several other EPEC strains expressing the LA phenotype (16), whereas hybridization with DA EPEC strains was not observed. Thus, the factors mediating LA and DA seem to be genetically distinct (17).

Here we report the cloning and expression of a DNA fragment from a 100-kb plasmid harbored by EPEC clinical isolate 2787 which confers the DA phenotype to recipient *E. coli* K-12 strains as well as the identification of an approximately 100-kDa protein as DA-mediating adhesion.

MATERIALS AND METHODS

Bacterial strains and plasmids. EPEC strain 2787 (O127:H27), originally isolated from a case of infantile diarrhea, was kindly provided by H. Einecke (Hygiene Institut, Universität Heidelberg, Heidelberg, Federal Republic of Germany) along with other enteritis isolates. Serotyping was performed by I. and F. Orskov (Statens Seruminstitut, Copenhagen, Denmark). Strain KS52 (serotype O2) was isolated from a patient with pyelonephritis and originally obtained from G. Kallenius (7). Strain KK88 was obtained from the Universitäts-Kinderklinik, Heidelberg.

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TABLE 1. Properties and sources of *E. coli* strains

Strain	Pattern of adherence to HeLa cells	Serotype	Source
KS52	DA	O2	Pyelonephritis
KK88		ND[a]	Enteritis
1321	DA	O44	Enteritis
2322	DA	O126	Enteritis
2787	DA	O127	Enteritis
3707	DA	O126	Enteritis
8065	DA	O55	Enteritis
9980	LA	O111	Enteritis
14084	LA	O55	Enteritis

[a] ND, Not determined.

The sources and serotypes of the bacterial strains used are summarized in Table 1. *E. coli* K-12 C600 (F$^-$ *thi-1 thr-1 leuB6 lacY1 tonA21 supE44* λ^-) was used as the host for all recombinant plasmids.

Bacteria were grown overnight at 37°C in liquid culture or on agar plates solidified with 1.5% agar (Difco Laboratories, Detroit, Mich.) with the appropriate antibiotics (tetracycline [25 μg/ml] and ampicillin [100 μg/ml]; Sigma Chemical Co., St. Louis, Mo.).

Plasmid DNA isolation and cloning techniques. Plasmid DNA was isolated essentially as described by Birnboim and Doly (2). Enzymes were used according to the specifications of the manufacturers or as recommended by Maniatis et al. (15). The DNA was electrophoresed in 0.8% agarose gels, stained with ethidium bromide, and visualized by UV illumination. Transformation procedures followed the protocol given by Mandel and Higa (14).

Isolation of restriction endonuclease-generated DNA fragments. DNA fragments generated by digestion with endonucleases were separated by electrophoresis in 0.8% agarose gels and stained with ethidium bromide. After excision of the respective bands, the DNA was electroeluted to DEAE paper. Specific DNA fragments were isolated from DEAE paper with 1.5 M NaCl in TE buffer (10 mM Tris hydrochloride [pH 8.0], 1 mM EDTA).

Adhesion assay. To test for adherence of bacteria to HeLa cells, the method described by Cravioto et al. (3) was modified slightly. In short, for each assay, about 10^8 bacteria grown overnight at 37°C in standard I medium (E. Merck AG, Darmstadt, Federal Republic of Germany) with aeration were incubated for 5 min in 1 ml of phosphate-buffered saline (PBS) containing 0.5% D-mannose. The bacterial suspension was added to HeLa cell monolayers grown overnight on glass cover slips just before the monolayers reached confluency. After 1 h of incubation at 37°C, the cells were washed extensively with PBS to remove nonadherent bacteria. The cells were fixed in 70% methanol, stained with Giemsa (10% solution in water) for better contrast, and evaluated for adhering bacteria by light microscopy.

Electron microscopy. Bacteria suspended in PBS were added to carbon-coated copper grids for 90 s and, after removal of excess liquid with filter paper, negatively stained with 1% uranyl acetate. Samples were examined in a Philips 400 transmission electron microscope.

Southern blotting. DNA fragments to be used as probes were separated by agarose gel electrophoresis and electroeluted to DEAE paper (5). The eluted DNA fragments were radioactively labeled with [α-^{32}P]dCTP by using a nick translation kit (Boehringer GmbH, Mannheim, Federal Republic of Germany). The isolated plasmid DNA to be probed was electrophoresed through a 0.8% agarose gel. The DNA was depurinated by being washed with 0.25 N HCl for 20 min, denatured with 0.5 M NaOH for 20 min, and neutralized with 0.5 M Tris hydrochloride (pH 7.5) containing 1.5 M sodium chloride for 20 min. The fragments were transferred overnight to nitrocellulose filters with 2× SSC solution (300 mM sodium chloride, 30 mM sodium citrate [pH 7.0]) by capillary action. The filter was incubated for 2 h at 80°C. For hybridization with the ^{32}P-labeled DNA fragment, the filter was preincubated for 1 h at 42°C in 50 mM sodium phosphate (pH 6.5) containing 50% formamide, 5× SSC, and 1× Denhardt solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin). The radiolabeled DNA probe was denatured by boiling in the prehybridization solution and then added to the nitrocellulose filters. Incubation continued at 42°C overnight. The filters were then washed under stringent conditions (50°C) twice with 2× SSC–0.1% sodium dodecyl sulfate (SDS) and twice with 0.1× SSC–0.1% SDS. The dry filters were autoradiographed with X-Omat film (Eastman Kodak Co., Rochester, N.Y.) for various times at −70°C.

Adhesin extraction. Surface-associated adhesin protein was solubilized by incubation of bacteria at 60°C for 20 min in 100 mM sodium phosphate (pH 7.0) (4).

SDS-PAGE. SDS-polyacrylamide gel electrophoresis (PAGE) was performed in a discontinuous buffer system by the method of Laemmli (10).

RESULTS

Characteristics of EPEC isolates. Several EPEC isolates as classified by clinical manifestation of disease as well as serotype (Table 1) were assayed for ability to adhere to HeLa cells in tissue culture. All strains exhibiting either LA or DA carried several large plasmids (see Fig. 5A). Strain 2787 (serotype O127:H27), isolated from a case of infantile diarrhea, exhibited DA to HeLa cells and was therefore used in our study. Expression of the DA phenotype in strain 2787 was independent of growth conditions. We could not detect any fimbriae either by electron microscopy of negatively stained *E. coli* 2787 (Fig. 1) or by shearing of DA-positive recombinants and concentration of the supernatant, a method frequently used for detection and isolation of pili (6). Strain 2787 did not agglutinate human, rat, rabbit, mouse, or duck erythrocytes and proved negative for agglutination of latex beads absorbed with D-mannose or synthetic α-D-galactosyl-(1,4)-β-D-galactose (p specificity). *E. coli* 2787 contained three plasmids, one of about 3 kb and two large plasmids of about 100 kb.

Since the genes coding for expression of LA mediated by EAF have been localized on a large plasmid (1), we screened the extrachromosomal elements of strain 2787 for genes responsible for expression of DA.

DA to HeLa cells. To identify transformants expressing the DA phenotype, we screened for adherence of bacteria to HeLa cells. The adhesion assay was performed at 37°C in PBS in the presence of 0.5% D-mannose for 1 h. Under the conditions used in our assay, the clinical isolate 2787 adhered strongly to HeLa cell monolayers (Fig. 2), in contrast to C600 harboring pBR322, which did not adhere. Further incubation of the bacteria with HeLa cells at 37°C did not lead to a change in the attachment pattern, in contrast to the results described by Vial et al. (23). Strain 2787 did not show an aggregative pattern of adherence after prolonged incubation with HeLa cells.

Cloning of a plasmid-derived DNA fragment responsible for expression of DA. To identify and clone plasmid-encoded

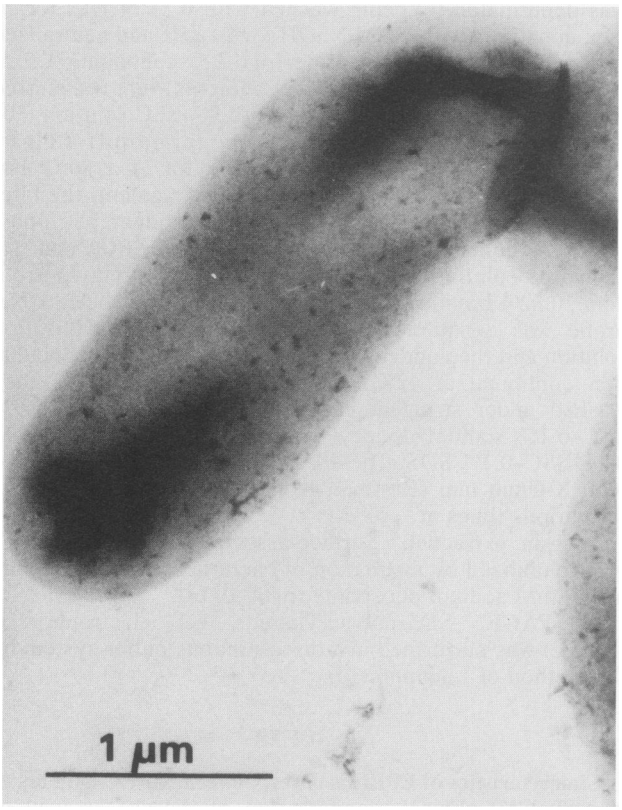

FIG. 1. Electron micrograph of the *E. coli* wild-type isolate 2787 grown in standard I liquid medium and stained with 1% uranyl acetate.

genes responsible for DA, total plasmid DNA isolated from *E. coli* 2787 was partially cleaved with *Eco*RI. The resulting fragments had sizes of between 10 and 20 kb and were exclusively derived from the two large 100-kb plasmids, since the small 3-kb plasmid did not contain an *Eco*RI restriction site. These fragments were ligated to *Eco*RI-digested and dephosphorylated pBR322, which served as the cloning vector. After transformation of *E. coli* K-12 C600, about 600 ampicillin-resistant transformants were screened for functional expression of an adhesin mediating DA by assays for the ability to attach to HeLa cells. One DA-positive clone was identified and found to harbor a plasmid designated pIB6. This plasmid contained an 11-kb DNA insert able to confer the DA phenotype to *E. coli* K-12 strains (Fig. 2).

Subcloning of DA-expressing DNA fragments. To define the smallest DNA fragment coding for functional expression of the DA phenotype, a linear restriction map of pIB6 (Fig. 3) was generated and further used for construction and analysis of deletion mutations in the 11-kb insert carried by pIB6 (Fig. 4). When assayed in the HeLa cell adhesion system, only three of the constructs, pIB4, pIB7, and pIB264, were still able to confer the DA phenotype to C600 (Table 2). pIB4 was derived from a deletion of the 1.7-kb *Cla*I fragment, whereas pIB7 was constructed by deleting a 1.5-kb *Pvu*I fragment that included 624 base pairs of vector DNA. pIB264 contained the 6.0-kb *Sph*I-*Cla*I fragment of pIB6. These results show that the 6.0-kb DNA fragment flanked by the *Sph*I and *Cla*I sites contained sufficient information for functional expression of the DA phenotype exhibited by the parent strain, 2787.

Location of genes expressing DA on plasmids harbored by *E. coli* 2787 and other EPEC isolates. Each of the clinical EPEC isolates used in this study harbored several plasmids

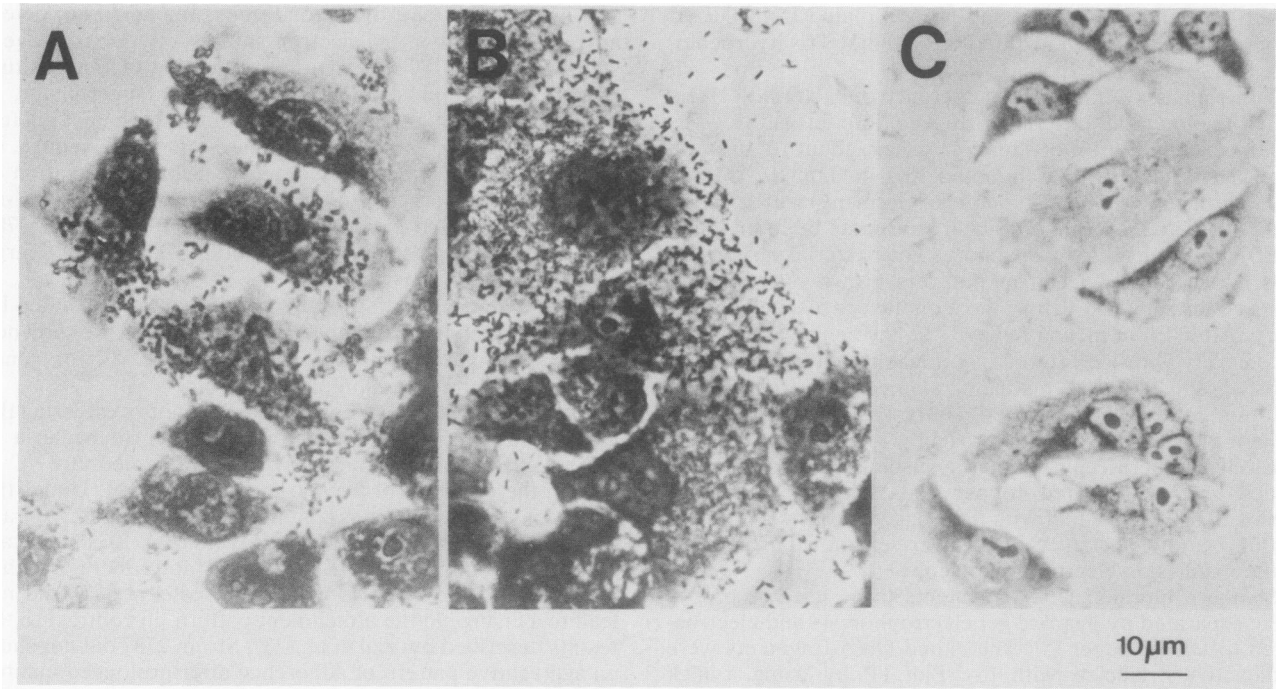

FIG. 2. Adherence of *E. coli* 2787 (A), C600(pIB6) (B), and C600(pBR322) (C) to HeLa cells in tissue culture. HeLa cell monolayers were incubated with 10^8 bacteria in PBS at 37°C for 1 h in the presence of 0.5% (wt/vol) D-mannose. After extensive washing with PBS, cells and bacteria were stained with Giemsa for better contrast and assayed for adherence by light microscopy.

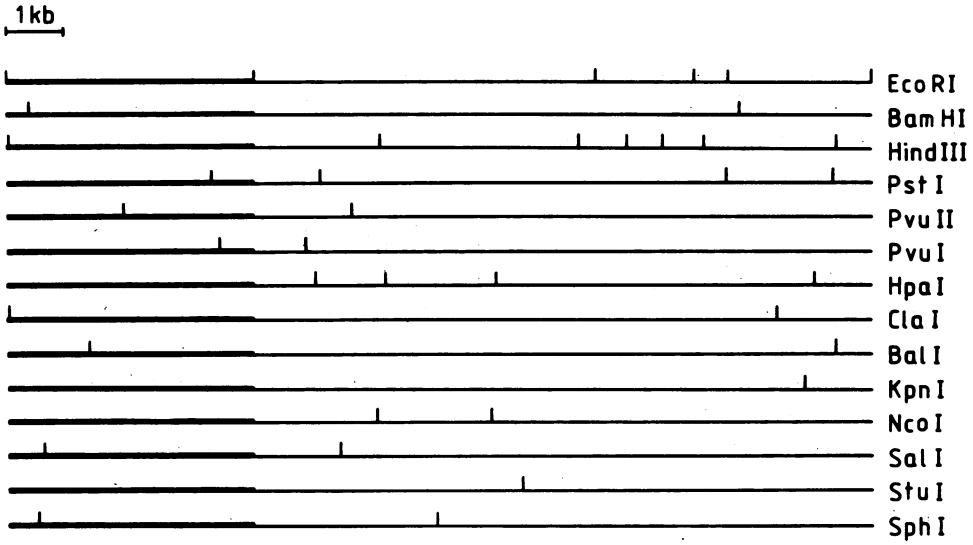

FIG. 3. Linear restriction map of recombinant plasmid pIB6. Symbols: ▬, pBR322 DNA; ——, insert DNA.

(Fig. 5A). We wanted to locate the genes responsible for expression of DA on a particular plasmid among the three harbored by the parental strain, 2787, as well as on corresponding plasmids of other EPEC strains exhibiting DA. Plasmids were purified from strain 2787 as well as from other isolates listed in Table 1 and electrophoresed through 0.8% agarose gels. After transfer to nitrocellulose, the plasmids were hybridized with the in vitro-^{32}P-labeled, nick-translated internal 3.5-kb *Hin*dIII DNA fragment of pIB6. Binding to homologous sequences was revealed by autoradiography (Fig. 5B). However, none of the isolated plasmids of heterologous DA- or LA-positive EPEC isolates hybridized with the *Hin*dIII probe except the largest of the three plasmids carried by the parental strain, 2787. Therefore, the genes coding for DA in strain 2787 were located on the larger of the two approximately 100-kb plasmids. None of the other DA-positive EPEC strains tested so far carries plasmids containing sequences homologous to those of the DNA fragment used as a probe. The same results were obtained when the four small *Hin*dIII DNA fragments were used for hybridization (data not shown). Since two of the plasmid preparations tested were derived from LA-expressing EPEC strains, the adhesin mediating DA in strain 2787 is genetically distinct from the adhesin supporting LA.

Detection of the adhesive protein mediating DA by SDS-PAGE. After cloning the genes encoding DA on a multicopy plasmid, we wanted to determine whether the adhesin was now produced in sufficient amounts to be detectable by SDS-PAGE. Outer membrane-associated proteins of transformants carrying plasmids pIB3, pIB6, pIB12, pIB264, and pBR322 were separated and analyzed by SDS-PAGE together with the wild-type isolate (Fig. 6). Comparison of the protein patterns exhibited by DA-expressing and -nonexpressing mutants showed only one difference: a faint, slightly diffuse protein band migrating at approximately 100 kDa was present only in DA-expressing mutants. This result indicated that the 100-kDa protein was representative of the adhesin involved in diffuse adherence, designated AIDA-I.

DISCUSSION

The aim of this study was to characterize the DA properties of the clinical EPEC isolate 2787 and to clone and express the genes responsible for this adherence pattern in

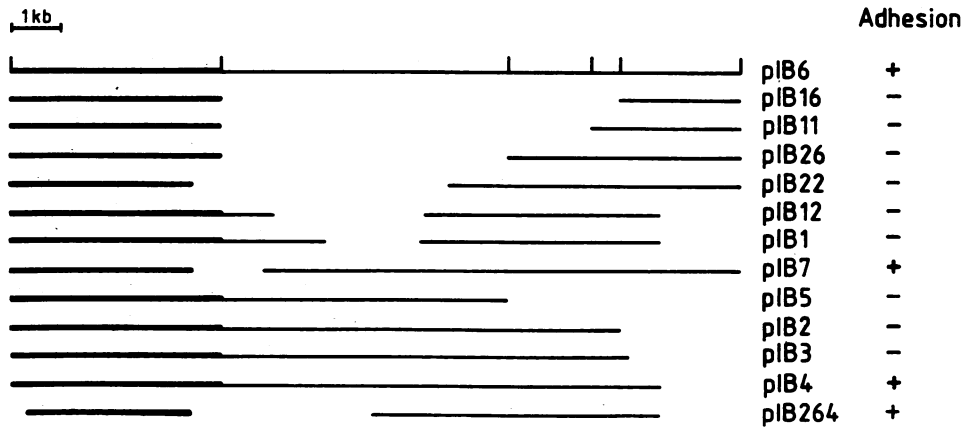

FIG. 4. Deletion analysis of pIB6 insert DNA by digestion with restriction enzymes. Symbols: ▬, pBR322 DNA; ——, insert DNA. Adherence phenotypes of transformants containing recombinant plasmids carrying deletions are indicated on the right.

TABLE 2. pBR322 hybrid plasmids and their properties

Plasmid	Phenotype		Insert size (kb)	Origin of insert
	Resistance	Adhesion		
pIB1	Amp^r	DA$^-$	7.2	pIB4 restricted with *NcoI*
pIB2	Amp^r Tet^r	DA$^-$	7.7	Partial *EcoRI* digest of pIB6
pIB3	Amp^r	DA$^-$	8.6	pIB6 restricted with *BamHI*
pIB4	Amp^r	DA$^+$	9.2	pIB6 restricted with *ClaI*
pIB5	Amp^r Tet^r	DA$^-$	6.0	Partial *EcoRI* digest of pIB6
pIB6	Amp^r Tet^r	DA$^+$	11.0	Partial *EcoRI* digest of 2787
pIB7	Tet^r	DA$^+$	10.0	pIB6 restricted with *PvuI*
pIB11	Amp^r Tet^r	DA$^-$	3.1	Partial *EcoRI* digest of pIB6
pIB12	Amp^r	DA$^-$	5.8	pIB4 restricted with *HpaI*
pIB16	Amp^r Tet^r	DA$^-$	2.5	Partial *EcoRI* digest of pIB6
pIB22	Tet^r	DA$^-$	6.2	pIB7 digested with *PvuI* and *StuI*
pIB26	Amp^r Tet^r	DA$^-$	4.9	Partial *EcoRI* digest of pIB6
pIB264	Amp^r	DA$^+$	6.0	*SphI-ClaI* fragment of pIB6

E. coli K-12. In our hands, this strain attaches to HeLa cells in a truly diffuse adherence pattern and does not adhere in the aggregative pattern reported by Vial et al. (23). We have shown that *E. coli* 2787 produced a specific, nonfimbrial, mannose-resistant adhesin which mediated adhesion to HeLa cells. Strain 2787 failed, however, to agglutinate human as well as rat, rabbit, mouse, and duck erythrocytes. The adhesive properties expressed by strain 2787 are different from those mediated by the EAF reported by Cravioto et al. (3). EAF supports a localized pattern of adherence, with formation of microcolonies, whereas 2787 exhibits a diffuse pattern upon incubation with HeLa cells. We believe, therefore, that 2787 is a representative of the group of EPEC distinguished by expression of the DA phenotype. An

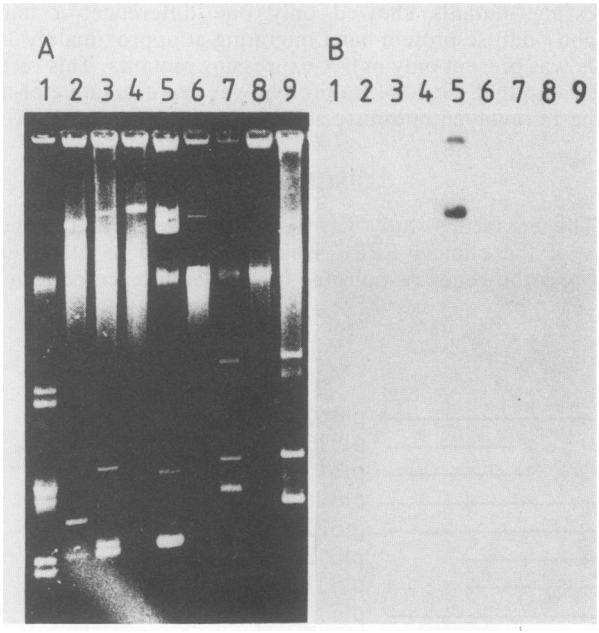

FIG. 5. Search for homologous plasmid DNA between the pIB6 DNA insert and large plasmids harbored by other EPEC isolates. Lanes: 1, KS52; 2, KK88; 3, 1321; 4, 2322; 5, 2787; 6, 3707; 7, 8095; 8, 9980; 9, 14084. Isolated plasmids were electrophoresed through a 0.8% agarose gel and visualized with ethidium bromide (A) or transferred to nitrocellulose and hybridized with a 3.5-kb *HindIII* in vitro-^{32}P-labeled, nick-translated DNA fragment isolated from pIB6 (B).

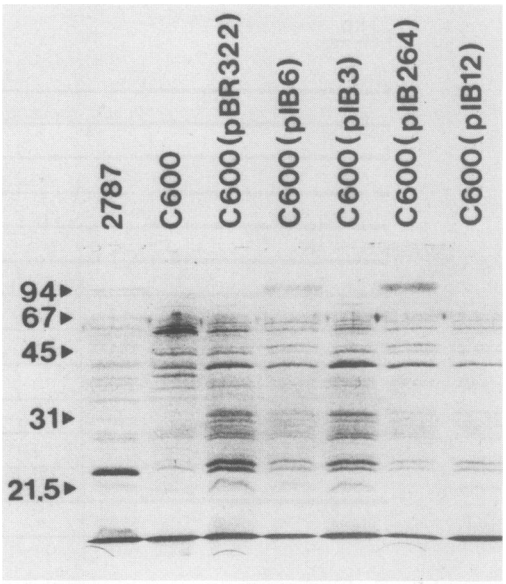

FIG. 6. Detection of AIDA-I by SDS-PAGE. Solubilized outer membrane-associated proteins were separated in a 12.5% SDS-polyacrylamide gel. The proteins were stained with Coomassie blue. Each lane was loaded with 800 ng of protein. Marker proteins had molecular sizes of 94, 67, 45, 31, 21.5, and 14.4 kDa (shown on left).

EPEC-derived nonfimbrial adhesin mediating diffuse adherence has not been described previously.

Our molecular cloning experiments revealed that the genetic determinants responsible for expression of the DA phenotype are located within a 6.0-kb DNA fragment originally derived from the larger of the two large plasmids harbored by the parental strain. This is reminiscent of the situation described for EPEC strains expressing the LA phenotype, as the genes coding for EAF have been identified on a ca. 100-kb plasmid designated pMAR2 (1). The size of the DNA fragment necessary for expression of LA, however, can so far be reduced only to about 20 kb (17). Plasmids containing the 6.0-kb DNA fragment (pIB4, pIB6, pIB7, and pIB264) confer the same diffuse adherence properties to C600 as occur in 2787. SDS-PAGE analyses of the protein patterns exhibited by C600 transformed with the DA-expressing plasmid pIB4, pIB6, or pIB264, in comparison with DA-negative deletion mutations as well as pBR322-carrying transformants, indicate that a protein of approximately 100 kDa, designated AIDA-I, is responsible for the DA properties. Thus, the size of AIDA-I is comparable to the 94 kDa reported for EAF by Levine et al. (13) but distinct from the 29 and 32 kDa reported for proteins by Scaletsky et al. (21); these findings suggest that in EPEC strains, proteins have evolved as adhesins that are different from the fimbrial or afimbrial adhesins described for extraintestinal pathogenic or intestinal pathogenic, heat-stable or heat-labile toxin-producing ETEC strains. Nonetheless, the adherence properties conferred on *E. coli* K-12 strains by genes coding for EAF and AIDA-I could be mediated by proteins that are of the same type but recognize different structures as receptors. The results from Southern hybridization experiments using the *HindIII* fragment of pIB6 as a probe with the group of available DA-expressing EPEC strains indicate that the adhesin conferring the DA phenotype may belong to a rather heterogeneous group of proteins that serve as virulence factors for EPEC. However, more restricted fragments should be used as hybridization probes, and many more

EPEC strains will need to be assayed for accurate assessment of the frequency of closely related proteins among DA-exhibiting EPEC strains. Further studies are in progress to determine the biochemical characteristics of AIDA-I and to identify the structure of its receptor on intestinal cells.

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LITERATURE CITED

1. **Baldini, M. M., J. P. Nataro, and J. B. Kaper.** 1986. Localization of a determinant for HEp-2 adherence by enteropathogenic *Escherichia coli*. Infect. Immun. **52**:334–336.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Cravioto, A., R. J. Gross, S. M. Scotland, and B. Rowe.** 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr. Microbiol. **3**:95–99.
4. **Darfeuille, A., B. Lafeuille, B. Joly, and R. Cluzel.** 1983. A new colonization factor antigen (CFA/III) produced by enteropathogenic *Escherichia coli* O128:B12. Ann. Microbiol. (Paris) **134A**: 53–64.
5. **Dretzen, G., M. Bellard, P. Sassone-Corsi, and P. Chambon.** 1981. A reliable method for the recovery of DNA fragments from agarose and acrylamide gels. Anal. Biochem. **112**:295–298.
6. **Hinson, G., S. Knutton, M. K.-L. Lam-Po-Tang, A. S. McNeish, and P. H. Williams.** 1987. Adherence to human colonocytes of an *Escherichia coli* strain isolated from severe infantile enteritis: molecular and ultrastructural studies of a fibrillar adhesin. Infect. Immun. **55**:393–402.
7. **Kallenius, G., R. Mollby, S. B. Svenson, J. Winberg, A. Lundblad, S. Svenson, and B. Cedergren.** 1980. The P^k antigen as a receptor for the haemagglutination of pyelonephritic *Escherichia coli*. FEMS Microbiol. Lett. **7**:297–302.
8. **Knutton, S., M. M. Baldini, J. B. Kaper, and A. S. McNeish.** 1987. Role of plasmid-encoded adherence factors in adhesion of enteropathogenic *Escherichia coli* to HEp-2 cells. Infect. Immun. **55**:78–85.
9. **Knutton, S., D. R. Lloyd, and A. S. McNeish.** 1987. Adhesion of enteropathogenic *Escherichia coli* to human intestinal enterocytes and cultured human intestinal mucosa. Infect. Immun. **55**:69–77.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Levine, M. M.** 1987. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J. Infect. Dis. **155**:377–389.
12. **Levine, M. M., E. J. Bergquist, D. R. Nalin, D. H. Waterman, R. B. Hornick, C. R. Young, and S. Stoman.** 1978. *Escherichia coli* strains that cause diarrhoea but do not produce heat-labile or heat-stable enterotoxins and are non-invasive. Lancet **i**: 1119–1122.
13. **Levine, M. M., J. P. Nataro, H. Karch, M. M. Baldini, J. B. Kaper, R. E. Black, M. L. Clements, and A. D. O'Brien.** 1985. The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an enteroadhesiveness factor. J. Infect. Dis. **152**: 550–559.
14. **Mandel, M., and A. Higa.** 1970. Calcium dependent bacteriophage DNA infection. J. Mol. Biol. **53**:159–162.
15. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Nataro, J. P., M. M. Baldini, J. B. Kaper, R. E. Black, N. Bravo, and M. M. Levine.** 1985. Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. J. Infect. Dis. **152**:560–565.
17. **Nataro, J. P., K. O. Maher, P. Mackie, and J. B. Kaper.** 1987. Characterization of plasmids encoding the adherence factor of enteropathogenic *Escherichia coli*. Infect. Immun. **55**:2370–2377.
18. **Nataro, J. P., I. C. A. Scaletsky, J. B. Kaper, M. M. Levine, and L. R. Trabulsi.** 1985. Plasmid-mediated factors conferring diffuse and localized adherence of enteropathogenic *Escherichia coli*. Infect. Immun. **48**:378–383.
19. **O'Brien, A. D., G. D. Laveck, M. R. Thompson, and S. B. Formal.** 1982. Production of *Shigella dysenteriae* type-1 like cytotoxin by *Escherichia coli*. J. Infect. Dis. **146**:763–769.
20. **Peeters, J. E., R. Geeroms, and F. Orskov.** 1988. Biotype. serotype, and pathogenicity of attaching and effacing enteropathogenic *Escherichia coli* strains isolated from diarrheic commercial rabbits. Infect. Immun. **56**:1442–1448.
21. **Scaletsky, I. C. A., S. R. Milani, L. R. Trabulsi, and L. R. Travassos.** 1988. Isolation and characterization of the localized adherence factor of enteropathogenic *Escherichia coli*. Infect. Immun. **56**:2979–2983.
22. **Scaletsky, I. C. A., M. L. M. Silva, and L. R. Trabulsi.** 1984. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. Infect. Immun. **45**:534–536.
23. **Vial, P. A., R. Robins-Browne, H. Lior, V. Prado, J. B. Kaper, J. P. Nataro, D. Maneval, A. Elsayed, and M. M. Levine.** 1988. Characterization of enteroadherent-aggregative *Escherichia coli*, a putative agent of diarrheal disease. J. Infect. Dis. **158**:70–79.