Introduction

Enteroaggregative *Escherichia coli* (EAEC) is an emerging diarrheal pathogen that has been associated with endemic and epidemic diarrheal illness both in developing and industrialized countries (1, 2). The pathogenesis of EAEC infection is thought to comprise adherence to the intestinal mucosa, most likely of both the small and the large intestines, followed by elaboration of one or more enterotoxins (3). These toxins include the heat stable toxin-like EAST1 and plasmid-encoded (Pet) toxin, the latter of which is responsible for mucosal cytotoxicity in explanted colonic tissue (1, 2, 4–6). Adherence of EAEC to the intestinal mucosa is characterized by the presence of a thick biofilm (2, 7), which may mediate persistence of this organism in the human intestine.

The defining feature of EAEC remains its distinctive aggregative adherence (AA) pattern observed in the HEp-2 adherence assay, in which the bacteria are coincubated with HEp-2 cells in cell culture medium for 3 hours. In this assay, EAEC adhere to the surface of HEp-2 cells, to the glass substratum, and to each other in a characteristic stacked-brick formation (8). We have shown that AA to HEp-2 cells in well-characterized EAEC strains requires a member of the aggregative adherence fimbriae (AAF) family (9, 10). AAF/I and AAF/II are each encoded on an approximately 100-kb virulence plasmid (designated pAA) (11, 12). The proven human pathogenic strain 042 requires AAF/II fimbriae for adherence to colonic explants (10).

Despite the importance of AAF fimbriae in adherence, we have observed that the majority of EAEC strains lack AAF/I and AAF/II (6). However, our data also suggest that most EAEC strains in our collection carry the pAA plasmid, as evidenced by the presence of several other conserved plasmid loci. Most prominent among these is a transcriptional activator of the AraC
class, designated AggR, which is required for expression of both AAF/I and AAF/II, but which is also present in a large percentage of EAEC strains that do not express any identified AAF. A second, cryptic locus (originally designated aspU) was found in 80% of EAEC strains in our collection (6). Here, we characterize the aspU locus, which lies immediately upstream of aggR in EAEC 042. This gene encodes a secreted low–molecular weight protein that appears to coat the bacterial surface and thereby promote dispersal of EAEC on the intestinal mucosa. Importantly, the protein, designated dispersin herein, corresponds to a previously cryptic protein against which EAEC-challenged volunteers demonstrated secretory IgA responses (9).

**Methods**

Cloning and molecular biology methods. Standard molecular biology techniques used published methods (13). *E. coli* DH5α was used as the host strain for recombinant DNA experiments unless otherwise noted (Table 1).

A mutation in the aap gene was constructed using single-crossover insertion mutagenesis as previously described (12). A DNA fragment internal to the aap gene was synthesized by PCR, and the fragment was cloned into the *KpnI* and *SacI* sites of suicide vector pJP5603 (14). Primers used to amplify the internal fragment had the following sequence, with restriction sites underlined: 5′-ATGGTACCTGTATCTTTCTGCGATCTTTGGT; 5′-ATGACCTGAGGGTTAACAACTCCTTTGATT.

A previously constructed aggR mutant strain, JS28, was complemented for AggR expression under control of the ara promoter in pBAD30. The aggR gene was amplified from 042 by PCR using the following primers: AggR-forward primer, 5′-GCCGGCATGTGAAATTTAAAAAAACATCGAAA-3′; and AggR-reverse primer, 5′-GCCGGTGTGGACCTTGGTTGAAAAATATTGCTAAG-3′. The product was digested with *SmaI* and cloned into the *SacI* site of expression vector pBAD30.

**PCR and RT-PCR for aap-aggR.** PCR was performed to assess the conservation of the aap-aggR locus using the following primers: Aap-forward, 5′-GCCCCGGGATGGAATTTAAAAAAACATCGAAA-3′; and AggR-reverse primer, 5′-GCCGGCTATTTGGTTTAAAAATATTGCTAAG-3′. RT-PCR reactions were performed from *E. coli* cultures as previously described (15). Primers used for cDNA synthesis were cat (5′-CCTCCTATCCGAGTAC-3′), aap (5′-TTATTTACCCCATCGGTAGAG-3′), and aggR (5′-GCCGGGATGGAATTTAAAAAAACATCGAAA-3′). For the PCR step, the reverse primers were as above, paired with the following forward primers: cat (5′-TACCTAGATTACCCCAAGT), aap (5′-ATGAAAAAATTAGTTTGTATAC-3′), and aggR (5′-GCCGGGATGGAATTTAAAAAAACATCGAAA-3′).

**Expression and purification of the aap gene product.** To facilitate purification of the aap gene product, the aap gene was ligated into the *Spbi*-BamHI sites of plasmid pQE70 downstream of the lac promoter. The resulting plasmid, pAap, expresses dispersin fused to six histidine residues at the C-terminus. The aap gene was amplified by PCR using primers with the following sequences: 5′-CAATGCGACAAAAATTAAGTTTTTGATACG, and 5′-CGGATGCCACCCATCTCGGTATAGGC. Amplification results in a DNA fragment with *SphI* and *BamHI* restriction sites at the aap upstream and downstream ends, respectively (underlined nucleotides in primers above). The PCR product was digested with *SphI* and *BamHI* and ligated into similarly digested vector pQE70.

The Aap-6His fusion protein was purified from a 100-ml culture volume grown in L-broth with shaking at 37°C to an OD₆₀₀ of 0.6. At this point, IPTG (Sigma Chemical Co., St. Louis, Missouri, USA) was added to a final concentration of 1 mM, and incubation at 37°C was continued for an additional 4 hours. Extraction of the protein was performed according to manufacturer’s protocol no. 10 (QIAexpress manual version 3/99; QIAGEN Inc., Valencia, California, USA).

Polyclonal antiserum was raised against the purified Aap-6His protein fusion by subcutaneous injection of rabbits with Aap protein excised from SDS-PAGE gels as described (16). We performed dot blots for AAF/II expression by spotting 100-µl aliquots from shaking L-broth cultures onto nitrocellulose paper. Blots were then processed using a 1:500 dilution of anti-AAF/II antiserum previously described, and then developed by standard methods using goat anti-rabbit IgG as previously reported (9).

**Localization of the aap gene product.** Overnight L-broth cultures were diluted 1:20 in 5 ml fresh L-broth and grown to log phase. The cells were pelleted for 15 minutes at 1,100 g, and both supernatant and pellet fractions were recovered. The supernatant was precipitated by addition of trichloroacetic acid (TCA) to final concentration of 20%. TCA precipitate was collected by centrifugation at 14,000 g. Loosely adherent surface protein was removed by (a) addition of Triton X-100 detergent (Sigma Chemical Co.) to the L-broth culture (final concentration 0.1%) 1 hour before harvesting of cells; (b) heating of the culture to 60°C for 20 minutes prior to separation of whole cells and supernatant; or (c) vortexing of a 5-ml culture in a VWR Vortex Genie (VWR Scientific, Bridgeport, New Jersey, USA) at the highest setting for 10 minutes.

**Adherence and autoagglutination assays.** The HEP-2 adherence assay was performed as previously described (17). In certain experiments, purified Aaf or BSA (Sigma Chemical Co.) was added to the cells in fresh medium immediately prior to the addition of the bacterial inoculum. Glass-adherence assays were performed as described (15). The autoagglutination assay was performed as described by Hasman et al. (18). Quantitation of adherent organisms was performed by triplicate plate count of serial dilutions removed by 10 minutes’ incubation in 1% Triton X-100.

**Mucus penetration assay.** Mucus penetration was measured by a modification of the method described by Lehker and Sweeney (19). Cells grown overnight in L-broth were diluted 1:200 in L-broth and grown to mid-log phase for 3 hours. Bacteria were pelleted by
centrifugation and resuspended in PBS (pH 7.4) to 1 × 10⁷ cells/ml. Bovine submaxillary mucin (Sigma Chemical Co.) was prepared to a concentration of 10 mg/ml in HBSS (GIBCO BRL; Life Technologies Inc, Gaithersburg, Maryland, USA.) and was loaded into a 1-ml tuberculin syringe fitted with a stopcock and 22-gauge needle, creating a mucous column. Ten microliters of cell suspension was gently layered onto the mucin, using a separate column for each strain. The column was incubated at 37°C for 75 minutes in an upright position. Fractions consisting of ten drops each were collected from the bottom of the column. The starting inoculum and each fraction were plated to L agar media with appropriate antibiotics to determine the number of CFUs per ml.

**In vitro organ culture.** The effect of the aap mutation was studied on colonic tissue using pediatric intestinal biopsies in an in vitro organ culture model as previously described (20). Histologically normal samples of transverse colon from six pediatric patients (five male, one female; aged 41–174 months; median age 107 months) were obtained during routine endoscopic examination after ethical approval and informed parental consent.

**Electron microscopy.** Immunogold transmission electron microscopy for detection of Aap was performed on overnight Luria broth cultures as previously described (10, 21). For scanning electron microscopy, strains were grown in L-broth overnight with shaking at 37°C. Thirty microliters of an overnight bacterial culture was spotted onto silica chips (Ted Pella Inc., Redding, California, USA) previously coated with 0.1% polylysine (Sigma Chemical Co.) and allowed to incubate at room temperature. Liquid was again withdrawn, and 0.1 M CaCo buffer was applied three times for 5 minutes per wash. The specimen was then post-fixed with 2% osmium tetroxide (Electron Microscopy Sciences Inc.) in CaCo buffer for 1 hour on ice. The specimens were rinsed twice with distilled water for 5 minutes each. Specimens were then stained with 2% uranyl acetate (Ted Pella Inc.) for 30 minutes at room temperature, rinsed with 50% ethanol, then dehydrated for 5 minutes each in sequential baths of 50%, 70%, 90%, and 100% ethanol. The 100% ethanol wash step was performed three times for 5 minutes each. The specimens were then inserted into a critical point dryer (model CPD-030; BAL-TEC AG, Balzers, Switzerland) until dry, followed by sputter coating in a Desk II Cold Sputtercoater for 90 seconds using platinum and paladium according to the manufacturer’s protocols (Denton Vacuum Inc., Moorestown, New Jersey, USA). Specimens were then examined by scanning electron microscopy in a LEO 1550 field emission scanning electron microscope (LEO Electron Microscopy Inc., Thornwood, New York, USA).

**Figure 1**
Map of aap-aggR loci from strains 042 (producing AAF/I) and 17-2 (producing AAF/I). The aap-aggR locus is highly conserved between the two strains, despite divergence of the fimbrial loci. The aap-aggR intergenic region is 99% identical in the two strains, except for a 50-nucleotide insertion present in the 17-2 sequence. IS, insertion sequence. Values percent indicate nucleotide homology between 042 and 17-2 sequences.

**Figure 2**
RT-PCR for aap transcript in 042, 042aggR, and 042aggR(pBADaggR). Cultures were grown to late log phase in either L-broth with 0.5% glucose or L-broth with 0.5% arabinose. RT-PCR was performed as described in Methods, and products were separated on 1% agarose gels. The aap transcript migrates as a 0.3-kb species as predicted.
Results

Characterization of the aap locus. We have previously reported that aggR, encoding a transcriptional activator of AAF expression, is encoded downstream of the fimbrial subunit (aafA) on pAA2 in prototype EAEC strain 042 (Figure 1). Sequencing of plasmid DNA upstream of aggR revealed an open reading frame (ORF) terminating 815 nucleotides from the aggR start codon. The ORF was 348 nucleotides in length, encoding a predicted protein product of 116 amino acids. SignalP analysis strongly predicted a signal sequence with cleavage after position 21. The predicted N-terminal amino acid sequence of the mature species was identical to the N-terminus of a cryptic protein we had previously reported to be secreted from EAEC strain 17-2, and initially surmised to be a fimbrial subunit (ref. 9; GenBank accession no. A49221). The predicted mature protein product of aap is 10.2 kDa in size. Neither the nucleotide sequence of aap nor its deduced amino acid sequence displays significant identity to any other protein or gene in the databases.

The aap-aggR region was remarkably conserved between AAF/I–encoding strain 17-2 (GenBank accession no. Z32523) and AAF/II–encoding strain 042 (Figure 1), despite the fact that the organizations of AAF/I and AAF/II biogenesis genes are dramatically different (12, 22). To assess the conservation of the aap-aggR locus among EAEC strains, PCR was performed on total genomic DNA from a selection of EAEC strains previously hybridized with an aggR probe (6). Primers were chosen from aggR and aap in strain 042 such that the PCR product would span the intergenic region. All strains known to hybridize with the aggR probe yielded identical 2-kb fragments; all probe-negative strains did not yield a PCR product (not shown).

Since aap and aggR are separated by less than 1 kb, we asked whether or not the two genes were transcriptionally linked. RT-PCR was performed using the aap upstream primer and the aggR downstream primer from the experiments above. This RT-PCR reaction did not yield a product, suggesting that the two are not transcribed polycistronically (not shown).

aap transcription is AggR-dependent. AggR is required for transcriptional activation of AAF/I and AAF/II genes, and its proximity to aap suggested that AggR might also be required for aap expression. To test this hypothesis, we performed RT-PCR for the aap transcript in strains 042 and 042 aggR (Figure 2). A product of the predicted size was seen in 042 but was absent in 042 aggR. To confirm a requirement for AggR, we complemented 042 aggR with aggR cloned under control of the arabinose-dependent promoter in pBAD30. Restoration of aap expression was observed in 042(pBAD aggR) when cells were grown in the presence of arabinose (ara-inducing conditions) but not when they were grown in the presence of glucose (repressing conditions).

Localization of the aap gene product. The aap gene product has a strongly predicted signal sequence, and previous
data have shown that the protein can be found in bacterial supernatants (9). However, quantitative immunoblot experiments suggested that most of the Aap protein pelleted with the bacterium. Experiments were performed to resolve this discrepancy (Figure 3). Broth cultures of 042 were heated to 60°C for 20 minutes or subjected to vigorous vortexing. Both heat and shear forces remove bacterial fimbriae (23). Heat treatment of the culture slightly increased the amount of protein found in the supernatant; vortexing did not release Aap. In contrast, addition of Triton X-100 to a final concentration of 0.1% slightly increased the amount of protein found in the supernatant; vortexing did not release Aap. In contrast, addition of purified Aap protein to 042aap resulted in partial rescue of the wild-type phenotype (Figure 4i).

Altered adherence and autoagglutination in an aap mutant. Because of its location near adherence-related genes in EAEC strains and the appearance of 042aap under scanning electron microscopy, we suspected that the aap gene product may play a role in EAEC autoagglutination and adherence. Indeed, stationary-phase broth culture of 042aap exhibited differences in spontaneous settling of bacterial aggregates to the bottom of the culture tube (Figure 5). This difference was reversed in 042aap(pAap) (Figure 5a). Increased aggregation of 042aap was confirmed by transmission electron microscopy (Figure 5b). Aggregation of bacteria in broth cultures was quantitated using the settling assay described by Hasman et al. (18). A typical result of this assay is depicted in Figure 6.

In the conventional 3-hour HEp-2 adherence assay, 042aap displayed dramatically larger aggregates than did its wild-type parent (Figure 7, a and b). This

**Figure 5**
Autoagglutination of 042 and mutants in L-broth. L-broth cultures were grown overnight at 37°C with shaking, then permitted to settle undisturbed at room temperature for 1 hour. (a) Broth cultures revealing a pellet at the bottom of the culture tube correlating with autoagglutination of bacterial cells. (b) Transmission electron microscopy of 042 and 042aap cultures. Ten microliters of L-broth cultures from a were withdrawn prior to incubation on the bench-top and processed for negative staining. Bars, 1 µm.

**Figure 6**
Spontaneous settling of 042 and mutants. Overnight L-broth cultures containing 0.2 mM IPTG were permitted to settle undisturbed at room temperature. At 15-minute intervals, a 100-µl sample was withdrawn from the top of each broth culture and the OD600 of the withdrawn sample was determined. Rate of clearing of the uppermost portion of the culture correlates with rate of spontaneous settling, a measure of agglutination. Samples were tested in triplicate. Bars represent SEM.
phenotype was also reversed in 042aap (pAap) (Figure 7c). When Aap protein was added to the HEp-2 adherence assay simultaneously with bacterial inoculation, adherence by 042aap returned to levels similar to those seen with wild-type (Figure 7d). Addition of BSA (Figure 7e) with the inoculum or addition of Aap after inoculation (not shown) did not have any effect on adherence. Quantitation of bacterial adherence by plate count revealed a dose-response relationship to IPTG concentration in 042aap (pAap), with two-log reduction in adherence upon addition of 0.05 mM IPTG. This concentration of IPTG does not affect growth rate of the test bacteria (not shown).

When a similar adherence assay was performed in the absence of HEp-2 cells (Figure 8), 042aap produced more abundant adherence to glass than did its parent, whereas adherence of 042aap (pAap) was similar to that of wild-type. Quantitation of this assay (Figure 8d) also revealed a dose-response relationship to IPTG concentration.

Roles for aap in mucosal adherence and mucus layer penetration. We have previously shown that EAEC strain 042 adheres abundantly to the surface of human intestinal biopsy sections in culture (24, 25). To investigate the role of the aap gene product in mucosal adherence, 042, 042aap, and 042aap (pAap) were incubated with

![Figure 7](http://www.jci.org)

Role of aap in epithelial cell adherence. Assays were performed for 3 hours in DMEM–0.5% glucose with 0.2 mM IPTG at 37°C in atmosphere. (a) 042; (b) 042aap; (c) 042aap (pAap). (d) and (e) The standard HEp-2 cell adherence assay was performed using strain 042aap, but with pure Aap (d) or pure BSA (e) added concurrently with the bacterial inoculum. Each protein was added to a final concentration of 0.1 mg/ml. (f) Quantitation of HEp-2 cell adherence on confluent monolayers growing in DMEM without IPTG (042 and 042aap) or with indicated IPTG concentration [042aap (pAap)]. Bacteria were removed from glass using 1% Triton X-100 and quantitated by viable count. Assays were performed in triplicate, and means are illustrated in histograms with error bars indicating SE. *P < 0.05 vs. 042 by one-tailed t test of log-transformed data; **P < 0.05 vs. 042aap by one-tailed t test of log-transformed data.

![Figure 8](http://www.jci.org)

Glass adherence assay. (a–c) Assay was performed for 3 hours in DMEM–0.5% glucose with 0.1 mM IPTG at 37°C in atmosphere. (a) 042; (b) 042aap; (c) 042aap (pAap). (d) Quantitation of adherence grown in DMEM without IPTG (042 and 042aap) or with the indicated concentration of IPTG ([042aap (pAap)]. Bacteria were removed from glass using 1% Triton X-100 and quantitated by viable count. Assays were performed in triplicate, and means are illustrated in histograms with error bars indicating SEM. *P < 0.05 vs. 042aap by one-tailed t test of log-transformed data.
fresh colonic biopsy specimens for 6 hours (Figure 9). Wild-type 042 adhered as previously described, producing a biofilm on the absorptive surface of the colonic mucosa, while sparing the colonic glands. Notably, 042 exhibited both aggregates and individual bacteria, very similar to the scanning electron microscopic images from silica chip experiments shown in Figure 4. In contrast, 042aap adhered in larger, tighter aggregates with very few single bacteria observed on the surface of the mucosa.

We hypothesized that decreasing the number of AAF-mediated aggregates could serve to facilitate the penetration of the 042 through the intestinal mucous layer, on the assumption that single bacteria would be more adept at swimming through the mucous gel. To test this hypothesis, we adopted a previously published method (19) in which bacteria are placed at the top of a column composed of purified mucin. These experiments revealed that 042aap was more than an order of magnitude less efficient at penetrating the mucin column than was the wild-type parent strain (Figure 10).

Secretion of Aap. Unresolved by the above experiments is the mechanism of Aap translocation past the outer membrane. We observed above that the predicted aap gene product possesses several attributes characteristic of bacterial pilin subunits, including a C-terminal glycine residue that mediates interaction with a PapD-like chaperone protein (26). We have previously shown that AafD and the PapC usher homolog AafC (12). To assess a possible role for the AafC/AafD secretion system in Aap expression, we assayed Aap in supernatants of 042 with insertional mutations in the aafD or aafC genes. Both mutants secreted Aap (Figure 11). Since aafD and aafC mutants are afimbriate (12), this experiment provides further verification that, though secreted, the Aap protein is not incorporated into the AAF organelle.

**Discussion**

We have previously shown that most EAEC strains in our collection harbor a partially conserved virulence plasmid (designated pAA), which typically encodes the transcriptional activator AggR. Here, we show that aggR is part of a highly conserved two-gene cluster, which also encodes a novel low–molecular weight secreted protein that mediates dispersal of EAEC on the colonic mucosa. In recognition of this phenotype, we have herein designated this gene aap (anti-aggregation protein). We now further suggest the more descriptive name “dispersin” for the aap gene product. Our data suggest that dispersin is secreted to the environment, but that it remains noncovalently associated with the bacterial cell surface.

Several roles for dispersin in adherence and colonization are plausible. All enteric pathogens must adhere to the epithelial surface. But at the same time, it may be beneficial to negatively modulate adherence to permit dispersal across the epithelial surface. Knutton et al. have shown that the bundle-forming pilus of enteropathogenic E. Coli (EPEC) mediates both aggregation and dispersal, which occur sequentially (27). In this way, EPEC can adhere and multiply early in the pathogenetic sequence, thereby establishing a foothold on the epithelium, but the bacteria then disperse individual progeny, which are free to establish new foci of infection. This paradigm is intuitively beneficial and therefore may be a property of many other mucosal pathogens. Indeed, Benitez et al. have suggested that the Hap mucinase of Vibrio cholerae may serve as an enzymatic “detachase,” mutation of which results in increased density of bacterial colonization but also attenuation of virulence (28).

We also hypothesized that dispersin may play a role in penetration of the intestinal mucous blanket, expecting that large aggregates would be retarded in translocation through a viscous gel. Indeed, using a purified mucin gel column assay, we showed that the dispersin mutant penetrated more slowly than the wild-type parent. The human colonic mucosa is covered with a continuous mucous gel approaching 1 mm in thickness.

**Figure 9**

In vitro organ culture assay of 042 (a) and 042aap (b). Bacterial cultures were incubated with colonic biopsies from normal children for 6 hours; then specimens were fixed, stained, and examined by scanning electron microscopy. White arrows (a) denote loose adherence of 042; gray arrows (b) show tight aggregates typical of 042aap. Bars, 10 μm.

**Figure 10**

Penetration of 042 and 042aap through mucus. Columns of 10 mg/ml mucin in HBSS were prepared in tuberculin syringes, and the bacterial inocula were applied to the top of the column. The columns were incubated at 37°C for 75 minutes. After this time, the mucin suspension was drained from the column in 0.1-ml fractions, and the number of 042 (black bars) or 042aap (white bars) was determined by quantitative plating in triplicate. Bars represent SD. Fraction 10 represents the bottom of the column, fraction 1 the top. *P < 0.05 by Student’s t test of log-transformed data.
(29), which provides a formidable barrier against colonic pathogens. Single bacteria may be more adept at swimming through this layer; alternatively, biophysical characteristics conferred by the dispersin protein coat could promote mucous layer penetration. Whatever their precise roles, Aap, Hap, and some component of bundle-forming pili are apparently the first examples of bacterial “dispersins,” which may facilitate efficient colonization via bacterial dispersal. Thorough elucidation of the roles of these dispersins in vivo has not yet been accomplished, because of the limited availability of good whole-animal models, but is clearly worthy of investigation.

We can propose one potential mechanism by which dispersin may promote dispersal. AAF fimbriae are highly hydrophobic (10), and this hydrophobicity would favor strong autoagglutination in an aqueous environment. The predicted amino sequence of dispersin is more hydrophilic than that of AAF/II, and thus the presence of dispersin on the surface of the bacterial cell may ameliorate surface hydrophobicity. Scanning electron microscopy data are consistent with this hypothesis. Absence of dispersin results in striking alteration in the morphology of the AAF fimbriae. In the absence of dispersin, the fimbriae cling to the surface of the bacterial cell, although their number and diameter do not appear changed. This would imply that the surface of the outer membrane under the dispersin coat is largely hydrophobic; such hydrophobicity could be mediated by outer membrane proteins, or perhaps by other hydrophobic surface structures previously described in EAEC (30).

Notably, despite the fact that aap is coregulated with AAF adhesins and that dispersin has C-terminal characteristics consistent with secretion by the usher-chaperone pathway, we found that mutants in either the usher or the chaperone secreted normal levels of dispersin. Thus, the mechanism by which dispersin is translocated across the outer membrane is as yet unknown. We found it interesting that AAF mutants bound less dispersin protein in the presence of Triton X-100. Immunofluorescence microscopy experiments suggest that some dispersin is bound within AAF-mediated aggregates (not shown), providing one possible explanation for this observation.

The requirement for AggR in aap expression is highly significant. AggR has been shown to be required for expression of AAF biogenesis genes (31), yet there are many strains that carry aggR and do not have an identified AAF adhesin (6). Our data thus suggest that AggR acts as a more versatile regulator of virulence

![Figure 11](http://www.jci.org/)

**Figure 11**
Western immunoblots for Aap performed on supernatants (S) or pellets (P) of overnight L-broth cultures with or without 0.1% Triton X-100. Positions of molecular weight markers are shown at right.

### Table 1
Strains and plasmids used in this work

<table>
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<tr>
<th>Strains</th>
<th>Characteristics</th>
<th>Reference</th>
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<tr>
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<td>Wild-type EAEC strain of proven pathogenicity</td>
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<td>042aap</td>
<td>042 harboring pJP5603 integrated into the aap gene (Km)</td>
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<td>44-1, 6-1, 17-2, 236, 199-1, 103-1, 101-1, 1096, 43996, DS-61R2</td>
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**Plasmids**

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<td>AggR cloned into multiple cloning site of pBAD30 to permit expression of aggR in the presence of arabinose</td>
<td>This work</td>
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Ap, ampicillin-resistant; Km, kanamycin-resistant.
genes in EAEC. Indeed, recent data from our laboratory suggest that AggR activates additional unlinked genes on the pAA plasmid and the EAEC chromosome (J. Nishi et al., unpublished results; and E. Dudley et al., unpublished results). Additional characterization of the AggR regulon and its role in EAEC pathogenesis is underway. Coexpression of AAF and dispersin in a common AggR regulon is consistent with an interactive role of the two factors in colonization, but it is interesting that the bacterium would use the same regulator to express both an adhesin and a negative modulator of adhesion.

EAEC is an emerging pathogen, but vaccine development is in its earliest stages. Several obstacles must be overcome before a candidate vaccine can be constructed. Chief among these obstacles is the antigenic heterogeneity of EAEC strains. In addition, given that EAEC infection is a serious health concern predominately in developing countries, there is little economic incentive toward the development of a vaccine. Therefore, the best approach to EAEC vaccine development would be to identify a highly conserved, highly prevalent, surface-exposed and immunogenic protein that could be expressed in an attenuated vector vaccine. The ideal vector would be one engineered to provide protection against another, perhaps more serious threat (such as shigellosis or typhoid fever). Dispersin fulfills many of these criteria, and experiments are underway to test its feasibility as an EAEC immunogen.

Acknowledgments
This work was supported by NIH grant AI-33096 to J.P. Nataro. We thank James Kaper for critical review of the manuscript and Alya Raphael for excellent technical assistance.