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Plasmid and Chromosomal Elements Involved in the Pathogenesis of Attaching and Effacing *Escherichia coli*

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Attaching and effacing (A/E) intestinal lesions are produced by enteropathogenic *Escherichia coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and RDEC-1, a pathogen of weanling rabbits. We recently identified a chromosomal locus (*eae* [*E. coli* A/E]) which is required for A/E activity in a wild-type EPEC strain. Sequences homologous to those of an *eae* gene probe were detected in EPEC, RDEC-1, and EHEC isolates. We report here that the *eae* gene is chromosomally encoded in all EPEC and EHEC strains tested and in RDEC-1. In addition, the *eae* probe was found to be 100% sensitive and 98% specific in detecting *E. coli* of EPEC serogroups that demonstrate A/E activity. Ten percent of *E. coli* of EPEC serogroups that hybridized with the *eae* probe and produced A/E activity did not hybridize with the EAF (EPEC adherence factor) probe, a plasmid-associated diagnostic probe which is currently used to identify EPEC. In addition to A/E factors, plasmid-associated adhesins also contribute to the pathogenesis of EPEC and RDEC-1. To further investigate the role of plasmid-associated adherence, a hybrid RDEC-1–EPEC strain containing the adherence plasmid of an EPEC strain in the A/E background of RDEC-1 was constructed. This hybrid strain, unlike the parent RDEC-1 strain, produced A/E lesions on human tissue culture cells, which suggests that the EPEC adherence plasmid provides tissue specificity to the hybrid strain and that the A/E factors of RDEC-1 are not host restricted.

Three classes of diarrheagenic *Escherichia coli* interact with the intestinal mucosa to produce a characteristic histopathology referred to as an attaching and effacing (A/E) cytoskeletal lesion. Enteropathogenic *E. coli* (EPEC), a major pathogen of infants in the developing world (8, 14, 15), produces A/E lesions in infected infants (43, 51, 54), in experimental animals (33, 42, 53), and on tissue culture cells (22, 23). A/E lesions are also produced by *E. coli* which are diarrheagenic for suckling and weanling rabbits (40, 41); the prototype of this class is strain RDEC-1 (50). More recently, it has been shown that isolates of enterohemorrhagic *E. coli* (EHEC), a cause of hemorrhagic colitis and hemolytic uremic syndrome in humans (26), produces A/E lesions in animals (46, 53) that are identical to those produced by EPEC and RDEC-1. EPEC, EHEC, and RDEC-1 are collectively referred to as the A/E *E. coli* (AEEC).

A/E histopathology is characterized by the close attachment of the bacterium to the enterocyte, effacement of the microvilli, disruption of the cellular cytoskeleton at the site of attachment, and "cupping" of the enterocyte membrane around the bacterium (33). High concentrations of filamentous actin are found in the host cell beneath the bacteria, a characteristic which is utilized in the fluorescent-actin staining (FAS) assay to detect the presence of A/E lesions on tissue culture cells (23). The FAS assay, which has been proposed as a rapid screening assay for the diagnosis of EPEC infection, is based on the use of fluorescein-isothiocyanate-labeled phalloidin to visualize the high concentrations of filamentous actin present in the A/E lesion.

The AEEC can be distinguished from each other by (ii) the serogroup to which the strain belongs (26), (ii) the production of high levels of Shiga-like toxin by EHEC but not by EPEC or RDEC-1 (26), and (iii) the presence of a high-molecular-weight plasmid associated with adherence that is different in each class. RDEC-1 carries a high-molecular-weight plasmid that encodes fimbriae called AF/R1 fimbriae, which mediate adherence to isolated rabbit brush border (56). The adherence plasmid of EPEC strains is called the EAF (EPEC adherence factor) plasmid and is associated with the ability of EPEC to adhere to tissue culture cells in a pattern called localized adherence (1, 39). The localized-adherence phenotype is characterized by the adherence of clusters of bacteria (referred to as microcolonies) to localized regions of tissue culture cells such as HEp-2 or HeLa cells (45). EPEC isolates cured of the EAF plasmid lose the ability to exhibit localized adherence to HEp-2 cells, and the introduction of this plasmid into laboratory *E. coli* host strains results in the acquisition of this phenotype by these strains (1). A 1-kb DNA fragment called the EAF probe was cloned from a region shown to be essential for the expression of localized adherence of one such plasmid (2) and is 99 to 100% sensitive and specific in identifying EPEC isolates that demonstrate localized adherence to tissue culture cells in vitro (15, 36, 39). EHEC also carry a large (ca. 60-MDa) plasmid that is associated with the presence of fimbriae (21), but the contribution of this plasmid to bacterial adherence is controversial. One study indicates that this plasmid mediates adherence (21), but another study failed to find a role for this plasmid in adherence (20).

Another important difference between EPEC, RDEC-1, and possibly EHEC strains may be the tissue specificity of these plasmid-encoded adhesins. Both EPEC and EHEC are human pathogens, yet EPEC, unlike EHEC, is a pathogen exclusively of young infants (26). RDEC-1, unlike EPEC and EHEC, is a pathogen of young rabbits and does not demonstrate in vitro adherence to isolated human brush border (7) or to tissue culture cells of human origin such as HeLa and

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Caco-2 cells (6; see below). It has also been shown that RDEC-1 adheres to rabbit microvilli in vitro but does not significantly adhere to microvilli of rats, guinea pigs, or humans. The importance of this host specificity is reflected by the ability of RDEC-1 to heavily colonize the intestine and to cause diarrhea in rabbits but not in rats or guinea pigs (7).

A common theme which has emerged from the study of the pathogenesis of the AEEC is the roles of both plasmid and chromosomal factors in virulence. Studies using plasmid-cured strains of EPEC (24, 53) and EHEC (53) have demonstrated that although the colonization plasmids of EPEC and EHEC may be involved in initial adherence to the brush border, they are not required for A/E activity. Similarly, Wolf et al. (56) demonstrated that a derivative of RDEC-1 inactivated in its ability to produce AF/R1 pili retained the ability to produce A/E lesions in rabbits. These studies suggest that chromosomal elements may mediate A/E activity in each of the AEEC. Although the genetics of A/E activity are not yet understood, we recently reported the identification of a chromosomal locus called *eae* (*E. coli* A/E) in EPEC E2348/69 that is necessary for the production of A/E lesions on tissue culture cells. Sequences homologous to a 1-kb probe from within the *eae* open reading frame were detected in other *E. coli* of EPEC serogroups that demonstrated A/E activity on HEp-2 cells. Interestingly, this probe also hybridized to RDEC-1 and the majority of EHEC isolates tested, suggesting genetic relatedness among the AEEC (19).

The purpose of the present article is twofold. First, we extend the previous study by Jerse et al. (19) to include the determination of the sensitivity and specificity of the *eae* probe in detecting isolates that demonstrate A/E activity in the FAS assay. The abilities of the chromosomal *eae* probe, the plasmid EAF probe, and the FAS assay to detect potential EPEC isolates were also compared. Second, in an attempt to study the tissue specificity of factors that mediate A/E activity, we constructed a hybrid RDEC-1–EPEC strain and evaluated this strain for its ability to produce A/E lesions on human tissue culture cells.

MATERIALS AND METHODS

Bacterial strains and plasmids. Selected bacterial strains used in this study are listed in Table 1. In addition to those listed, a collection of 193 *E. coli* strains of the major EPEC serogroups (O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158) and 16 *E. coli* of the minor EPEC serogroups (O18a,c and O44) (27) were tested for hybridization with the EAF and *eae* probes. These strains were isolated from infants with diarrhea in Peru, Chile, Israel, the United States, and miscellaneous other places. The strains mentioned above were obtained from the culture collections of the Center for Vaccine Development, University of Maryland, Baltimore.

Construction of hybrid strains. Strain E36-1C was created by curing pCVD428 (Ap^r Cm^r Km^r) from strain E36-1 by growth in novobiocin (57) and then isolating colonies that were sensitive to ampicillin, chloramphenicol, and kanamycin. Hybrid RDEC-1–EPEC strain M34(pMAR7), control strain HS-4(pMAR7), and strain E36-1C(pMAR7) were constructed by conjugating strain JPN15(pMAR7) with strains M34, HS-4, and E36-1C, respectively. Strain M34(pMAR7) was isolated by selecting for progeny that demonstrated resistance to kanamycin and ampicillin, while strains HS-4(pMAR7) and E36-1C(pMAR7) were isolated by selecting for progeny that demonstrated resistance to nalidixic acid and ampicillin. Conjugation was performed by standard methods (47), and the presence of pMAR7 was confirmed in the hybrid strains by analyzing uncut plasmids and restriction enzyme digestion patterns from plasmids extracted from all strains on 0.7% agarose gels.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description
E2348/69	EPEC strain (O127:H7) isolated from outbreak in Taunton, England (29)
JPN15	Strain E23438/69 spontaneously cured of pMAR2 after passage through a volunteer fed E2348/69 (19)
JPN15(pMAR7)	Strain JPN15 with pMAR7 introduced by conjugation (19)
RDEC-1	O15:nm; prototype strain of AEEC of rabbits containing AF/R1 plasmid encoding pili that mediate adherence to rabbit intestinal brush border (50, 56)
M34	Derivative of RDEC-1 in which *afr* gene encoding AF/R1 pilus has been inactivated by insertion of Tn*5* (56)
E36-1	O114:H19; isolated from 12-mo-old child with diarrhea in Chile; Nal^r (this report)
E36-1C	Derivative of E36-1 cured 60-Mda plasmid, pCVD428; Nal^r (this report)
HS-4	Nal^r derivative of normal flora *E. coli* HS, previously shown to be avirulent in volunteers (28)
pMAR7	Tn*801* derivative of pMAR2, native EAF plasmid of strain E2348/69 (1)

DNA hybridization. The previously described 1-kb *eae* probe spanning the location of the Tn*phoA* insertion in mutant JPN15.36 (19) and EAF21, a 21-base oligonucleotide probe that is highly sensitive and specific in detecting localized adherent *E. coli* (18), was evaluated for the ability to hybridize with EPEC and other bacteria by colony blot hybridization with Whatman 541 filters as described elsewhere (32). The 1-kb *eae* probe was radiolabeled with [α-^{32}P]ATP by the random-priming method (11) and hybridized by using high-stringency conditions as described previously (34). EAF21 was end labeled with [γ-^{32}P]ATP (31) and hybridized as described previously (18). For those strains which hybridized with the *eae* probe by colony blot, the location of the *eae* gene was determined to be plasmid or chromosomal by hybridizing the *eae* probe to plasmid DNA extracted by the method of Birnboim and Doly (4), separated on a 0.7% agarose gel, and transferred to nitrocellulose paper (48).

Tissue culture assays. The HEp-2 cell adherence assay was done as described previously (37), and the FAS assay was performed on HEp-2 cells (23) after 3 or 6 h of incubation with bacteria as indicated below. Caco-2 cells, a polarized human intestinal cell line (44), were incubated with bacteria for 8 h and processed for transmission electron microscopy as described previously (19).

RESULTS

Hybridization of *eae* probe to *E. coli* of EPEC serogroups. We previously examined 193 *E. coli* of the major EPEC serogroups and found that all 99 isolates that demonstrated A/E activity as determined by the FAS assay also hybridized with the 1-kb *eae* probe (19). In this report, we extend this study to include the *eae* hybridization results for the FAS-

TABLE 2. FAS assay and hybridization of *eae* and EAF probes to *E. coli* isolates

E. coli serogroup	Probe	No. of isolates		
		Tested	Hybridizing with *eae* probe	FAS positive[a]
Major EPEC[b]	EAF$^+$	89	89	89
	EAF$^-$	104	12	10[c]
O18a,c and O44	EAF$^+$	0	0	0
	EAF$^-$	16	0	0

[a] After 6 h of incubation with HEp-2 cells.
[b] O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158.
[c] These 10 isolates also hybridized with the *eae* probe.

TABLE 3. Adherence and A/E phenotypes of parent and hybrid strains carrying EAF plasmid pMAR7

Strain	HEp-2 cells		Caco-2 cells	
	Adherence[a]	FAS[b]	Adherence[c]	A/E lesion[d]
JPN15(pMAR7)	LA	+	+	+
E36-1	NA	−	−	−
E36-1C	NA	−	−	−
E36-1C(pMAR7)	LA	+	+	+
RDEC-1	NA	−	−	−
M34	NA	−	−	−
M34(pMAR7)	LA	+	+	+
HS-4	NA	−	−	−
HS-4(pMAR7)	LA	−	+	−

[a] LA, localized adherence after 3 h of incubation; NA, no adherence after 3 h of incubation.
[b] Results were determined after 3 and 6 h of incubation with HEp-2 cells.
[c] As determined by light microscopy.
[d] As determined by transmission electron microscopy.

negative isolates as well as the hybridization results for all 193 isolates tested with the EAF probe, which is specific for localized adherent *E. coli*. Eighty-nine of the 193 isolates hybridized with the EAF probe, and all were positive in the FAS assay (Table 2). The remaining 104 isolates did not hybridize with the EAF probe; however, 12 of these isolates hybridized with the *eae* probe. Ten of these 12 isolates were also positive in the FAS assay. On the basis of these data, the sensitivity and specificity of the *eae* probe in detecting *E. coli* which are positive in the FAS assay were 100 and 98%, respectively. The EAF probe, on the other hand, was 100% specific but only 90% sensitive in detecting FAS-positive isolates. Sixteen isolates of the less-common EPEC serogroups O18a,c and O44 were similarly tested. None of these isolates hybridized with the EAF or *eae* probes, and none were positive in the FAS assay (Table 2).

Location of the *eae* gene. Plasmids extracted from 77 *eae* probe-positive EPEC, 29 *eae* probe-positive EHEC isolates, and RDEC-1 were separated on 0.7% agarose gels and hybridized with the *eae* probe. Although high-molecular-weight plasmids were found in all of these strains, none of the strains carried a plasmid that hybridized with the probe. These data indicate that the *eae* locus is found on the chromosome of RDEC-1 and in all EPEC and EHEC strains examined.

Evaluation of hybrid RDEC-1–EPEC strain M34(pMAR7). To determine if the A/E factors of RDEC-1 might act on human tissue if RDEC-1 was provided with the EAF adherence plasmid of an EPEC strain, the hybrid RDEC-1–EPEC strain M34(pMAR7) was constructed. Strain M34 is a derivative of RDEC-1 which produces A/E lesions in rabbits but is insertionally inactivated in the *afr* gene encoding AF/R1 fimbriae (56). Unlike EPEC JPN15(pMAR7), strains RDEC-1 and M34 did not demonstrate localized adherence to HEp-2 cells after 3 h of incubation (Table 3). Similarly, neither RDEC-1 nor M34 produced a positive FAS result after 3 or 6 h of incubation with HEp-2 cells or A/E lesions on Caco-2 cells, in contrast to the positive FAS and A/E lesion results seen with JPN15(pMAR7) (Fig. 1A). As expected with the acquisition of pMAR7, the hybrid strain M34(pMAR7) exhibited localized adherence to HEp-2 cells. More interestingly, hybrid strain M34(pMAR7) produced a positive FAS staining pattern on HEp-2 cells after 3 h of incubation and A/E lesions on Caco-2 cells as seen by transmission electron microscopy (Fig. 1B). To control for the possibility that the plasmid pMAR7 mediates A/E activity, the plasmid pMAR7 was introduced into the normal flora strain HS-4 to create strain HS-4(pMAR7). Strain HS-4(pMAR7) exhibited localized adherence to HEp-2 cells, but unlike the hybrid M34(pMAR7), it produced a negative FAS result. Although light microscopy indicated that strain HS-4(pMAR7) adhered to the Caco-2 cells, transmission electron microscopy revealed that the bacteria were associated only with the brush border of the cells and that no characteristics of A/E histopathology were present (Fig. 1C).

E. coli E36-1 (O114:H19) is one of the two strains described in Table 2 that hybridized with the *eae* probe but did not hybridize with the EAF probe and did not demonstrate positive FAS activity on HEp-2 cells. To investigate the discrepancy between the *eae* probe and FAS results, the plasmid pMAR7 (Ap^r) was introduced into this strain to see if the presence of an EAF plasmid would enable strain E36-1 to exhibit A/E activity. Before introducing pMAR7 into strain E36-1, the strain was first cured of its 60-MDa native plasmid, pCVD428, as this plasmid was found to encode ampicillin resistance (as well as resistance to kanamycin and chloramphenicol). The EAF plasmid pMAR7 was introduced into the plasmid-cured, ampicillin-sensitive derivative E36-1C(Ap^s Km^s Cm^s) by conjugation. As expected, the resultant strain, E36-1C(pMAR7), demonstrated localized adherence to HEp-2 cells. Interestingly, in contrast to strains E36-1 and E36-1C, strain E36-1C(pMAR7) was positive in the FAS assay after 3 h of incubation with HEp-2 cells and produced A/E lesions on Caco-2 cells as seen by transmission electron microscopy (Table 3, Fig. 1D). As described above, the presence of pMAR7 in the normal flora strain HS-4 conferred the ability to produce localized adherence to HEp-2 cells but not the ability to produce A/E lesions.

DISCUSSION

Despite early descriptions of A/E histopathology (42, 49), the ability to produce A/E lesions has only gradually been accepted as a part of the definition of EPEC (9, 33). Over the last decade, increasing numbers of investigators have studied this aspect of EPEC pathogenesis by using animal models (33, 53) and cultured intestinal mucosa (3, 10, 24) in their analyses. The development of a rapid screening test for the A/E lesion, called the FAS assay by Knutton et al. (23), has helped document A/E activity as a characteristic of EPEC strains.

Using Tn*phoA* mutagenesis, we previously identified a chromosomal locus (*eae*) in an EPEC strain that is necessary for A/E activity on human tissue culture cells and reported

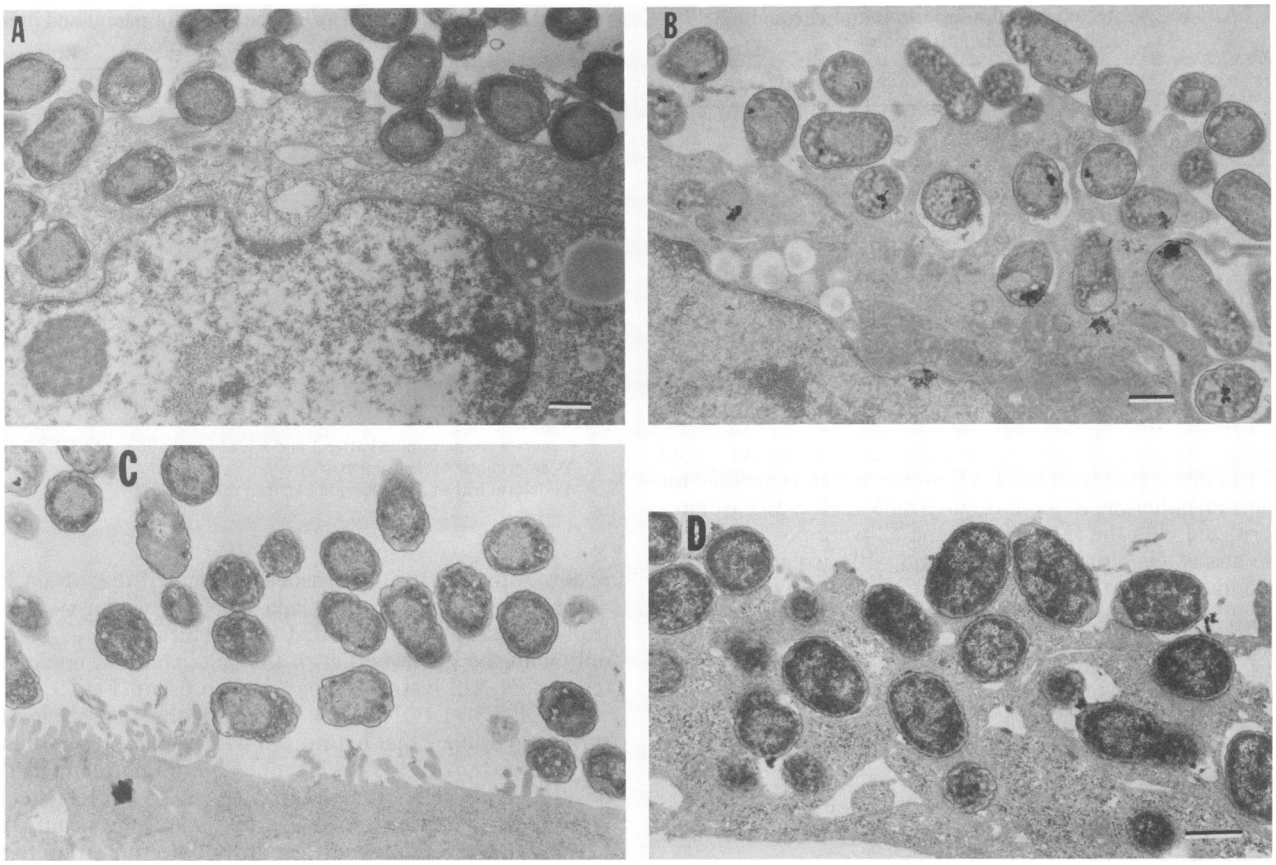

FIG. 1. Transmission electron micrographs of Caco-2 cells incubated with JPN15(pMAR7) (A), hybrid RDEC-1–EPEC M34(pMAR7) (B), strain HS-4(pMAR7) (C), and strain E36-1C(pMAR7) (D). Thin sections were examined with a Seimans IA or a JEOL 100B transmission electron microscope. Bar = 1 μm.

that a 1-kb probe from within the *eae* open reading frame hybridized to 100% of FAS-positive EPEC isolates (19). In the present study with 193 *E. coli* of EPEC serogroups, the *eae* probe was found to be 100% sensitive and 98% specific in detecting *E. coli* that demonstrate positive FAS activity on HEp-2 cells. The specificity of the probe may actually be higher than 98%, as evidenced by the fact that strain E36-1, which hybridized with the *eae* probe but was negative in the FAS assay, was able to produce A/E lesions when provided with the EAF plasmid pMAR7.

Although our knowledge of EPEC pathogenesis has significantly increased in recent years, the relative contribution of plasmid-encoded virulence factors versus chromosomally encoded factors remains unclear. The recognition of the plasmid-associated localized-adherence phenotype as a characteristic of EPEC strains and the subsequent development of the highly sensitive and specific EAF probe for detecting isolates that exhibit localized adherence have greatly aided epidemiological studies of EPEC. Studies that compare the isolation of EAF-positive *E. coli* of EPEC serogroups from cases versus controls support both the validity of the EAF probe in identifying EPEC isolates and the association of HEp-2 cell adherence with virulence (15, 30). The strictness with which EAF probe positivity and/or localized adherence to HEp-2 cells is to be considered a part of the definition of an EPEC, however, is not universally agreed upon (23). In the current study, we found that 10% of *E. coli* isolates that demonstrate A/E activity would have been overlooked as potential EPEC if the EAF probe had been the sole basis for identification. It has not yet been determined if such isolates (i.e., isolates that are localized-adherence negative and do not hybridize with the EAF probe but are positive in the FAS assay and hybridize with the *eae* probe) represent avirulent or weakly virulent *E. coli* or if they are EPEC strains that have lost the EAF plasmid during storage or upon passage through the host. The possibility that these isolates represent EPEC that are reduced in virulence is supported by human volunteer data which showed that the EAF plasmid was required for full virulence in adult volunteers (29). It should be noted, however, that spontaneous loss of the EAF plasmid occurred in 67% of the volunteers that received the wild-type strain, a finding which supports the possibility that EAF-negative, FAS-positive isolates may be EPEC strains that lost the EAF plasmid upon passage through the host. Although in vivo loss of the plasmid may be a phenomenon of the adult host, it may also occasionally occur in infants, as suggested by a study by Bower et al. (5) in which an EAF-negative *E. coli* isolate was recovered from 1 of 10 symptomatic children during an EPEC outbreak. This isolate was the same serotype as the EAF-positive isolates cultured from the other nine children in the outbreak. Loss of the EAF plasmid during storage may also occur, as suggested by a recent survey in which seven strains of classic EPEC serotypes associated with pediatric diarrheal outbreaks in the United States from 1954 through 1966 were reported not to hybridize with the EAF

probe or to demonstrate localized adherence (35). Finally, we cannot rule out the possibility that strains which hybridize with the *eae* probe but not the EAF probe may possess other plasmids, nonhomologous to the EAF plasmid, that serve a function similar to that of the EAF plasmid.

To investigate the significance of EAF-negative EPEC, we are currently evaluating the incidence of EAF probe-negative, *eae* probe-positive isolates in a prospective case-control study of childhood diarrhea in Santiago, Chile. Until the epidemiological significance of EAF probe-negative *E. coli* that produces A/E activity in vitro is known, however, it is perhaps advisable to screen potential EPEC isolates for A/E activity as well as for EAF probe positivity. The FAS assay was proposed by Knutton et al. (23) as a way of detecting EPEC isolates, regardless of the ability to demonstrate localized adherence to HEp-2 cells. A recent study (25) utilizing the FAS assay found a higher percentage of EAF probe-negative, FAS-positive strains (13 of 23) than we found in the present study (10 of 104). One significant difference between our study and that of Knutton et al. (25) is the source of the strains examined. Strains examined in the present study were isolated in numerous hospitals and clinics in Chile, Peru, Israel, and the United States, whereas strains examined by Knutton et al. were from a single hospital in London. The development of the highly sensitive and specific *eae* probe, with a positive predictive value of ≥98% and a negative predictive value of 100%, is more suitable for screening large numbers of strains than is the FAS test; the probe can be used to determine the incidence of AEEC in a variety of study populations.

It should be noted that no *E. coli* of serogroups O18a,c or O44 hybridized with the *eae* probe; neither have any strains of these serogroups been reported to hybridize with the EAF probe. Although serogroups O18a,c and O44 have been categorized on epidemiological grounds as EPEC serogroups, evidence which suggests that isolates of these serogroups are not EPEC is accumulating (23). Interestingly, the O44 serogroup has been associated with the newly described enteroaggregative *E. coli* (55).

The presence of sequences homologous to *eae* is not limited to EPEC strains, since homologous sequences are also found in other AEEC, namely EHEC and RDEC-1 (19). In the present study, we found that *eae* is chromosomally encoded in all strains examined, including 77 EPEC isolates, 29 EHEC isolates, and RDEC-1. Recent papers by Toth et al. (52) and Fletcher et al. (12) report the identification of native plasmids of an EHEC and an EPEC strain, respectively, that are capable of conferring A/E activity on laboratory *E. coli* host strains. In the report by Fletcher et al. (12), a high-molecular-weight plasmid in EPEC 22246 (O111:H$^-$) was able to confer A/E ability on *E. coli* DH1 in rabbit ileal explants, although at a much lower frequency than that produced by the wild-type strain. Although the *eae* probe hybridizes with the total DNA from strain 22246 in colony blot hybridization, it does not hybridize with any of the native plasmids found in this strain (16). If confirmed, the results of Fletcher et al. might suggest that another locus could substitute for the *eae* gene in producing A/E activity.

The presence of distinct colonization plasmids in the AEEC resembles the occurrence of distinct plasmid-encoded colonization fimbriae in enterotoxigenic *E. coli*. In light of this resemblance and the evidence that the colonization fimbriae of human and animal enterotoxigenic *E. coli* strains determine the host specificity of individual enterotoxigenic *E. coli* strains (26), we hypothesized that the plasmid-encoded adhesins of EPEC, RDEC-1, and EHEC isolates may dictate the tissue specificity of the AEEC. We addressed this hypothesis by using a hybrid strain carrying the EPEC EAF adherence plasmid in the A/E background of RDEC-1. Although RDEC-1 normally does not produce A/E activity on human cells (6), the presence of the pMAR7 plasmid enabled RDEC-1 to produce A/E lesions on human tissue culture cells. The exact mechanism(s) by which the EAF plasmid contributes to this phenomenon is not known. One possibility is that the EAF plasmid encodes an adherence factor which could bring A/E factors close enough to act. In support of this possibility, Francis et al. (13) have recently reported that type 1 pili and the afimbrial adhesin AFA-I can substitute for the EAF plasmid in the production of A/E lesions on human epithelial cells. Cantey et al. (6) have reported similar observations with the F1845 diffuse adherence factor added to RDEC-1. A second possibility is that the EAF plasmid provides a regulatory factor that acts on chromosomal A/E factors at the transcriptional level. Recent evidence from our laboratory suggests that the EAF plasmid contains a regulatory element capable of increasing expression of the *eae* gene (17). Finally, we cannot rule out the possibility that the plasmid encodes a protein which posttranslationally modifies the tissue specificity of the A/E factors.

In summary, we report that the *eae* gene of the AEEC is chromosomally encoded, and we demonstrate that the EAF adherence plasmid, in addition to enabling initial colonization, may provide tissue specificity for human epithelial cells. These findings confirm the existence of both plasmid-encoded and chromosomally encoded virulence factors in the AEEC. Although it is known that the EAF plasmid increases the virulence of an EPEC strain (24, 29, 53), the function of the plasmid sequences detected by the EAF probe has yet to be elucidated. The EAF probe sequences are highly conserved among those A/E *E. coli* that demonstrate localized adherence to HEp-2 cells (38), but it is not known whether these sequences are part of the DNA that encodes the actual adhesin. In contrast, the chromosomal *eae* gene is present in all AEEC and has been clearly shown to encode a membrane protein involved in production of the A/E lesion and to share significant homology to invasin, a known virulence factor of *Yersinia pseudotuberculosis* (17, 19). The role and importance of both chromosomal and plasmid-encoded elements in virulence needs to be addressed, as this information has implications in understanding the pathogenesis of EPEC as well as in the definition and diagnosis of this pathogen.

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