Mechanisms of Adherence of *Escherichia coli* to Enterocytes: Their Possible Role in Intractable Infant Diarrhea*

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Although the classical definition of intractable diarrhea of infancy (1), which includes three or more stools negative for bacterial pathogens, would tend to exclude a role for enteric infection in the pathogenesis of this form of diarrheal disease, a role for pathogenic *E. coli* may remain. The decade or more since this definition was proposed has seen tremendous advances in the laboratory recognition of pathogenic properties of *Escherichia coli* strains but no parallel advance in bringing these laboratory tests into clinical use. The fact remains that in most clinical laboratories, particularly those in developing countries, methods are not currently available to identify pathogenic *E. coli* in specific cases of diarrheal diseases. More often, tests for enterotoxin production, colonization factor antigens, and invasiveness of *E. coli*, or for the genes encoding for these properties, are only available in research laboratories or in centers performing epidemiological studies. The results are not often readily available to clinicians caring for sick children.

At the same time, screening *E. coli* strains for enteropathic serotypes has fallen into disuse because of a general feeling, unfortunately promoted by influential health organizations, that serotyping was unlikely to remain of clinical relevance with the development of more sophisticated laboratory tests reflecting more specific knowledge of pathogenic mechanisms. In part, serotyping has also fallen into disuse because of a real difficulty in identifying complete serotypes (including O, H, and K antigens) outside of the reference laboratory. Thus, at this time, even in the absence of positive stools for pathogens, *E. coli* should not be ignored, since they may yet emerge as having a significant role in this symptom complex.

Of particular interest in this context are mechanisms of adherence and colonization whereby *Escherichia coli* can establish themselves as more or less permanent residents at the mucosal surface (Fig. 1). Three types of pathological

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* The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.
interaction of *E. coli* strains with the intestinal mucosa have been described. These are exemplified by the enterotoxigenic *E. coli* (ETEC) (2–6), the entero-pathogenic *E. coli* (EPEC) (7–12), and the enteroinvasive *E. coli* (EIEC) (13–15). The first type of association, that of ETEC, is rather peripheral, with the organisms remaining at least 30 to 50 nm from the epithelial cell surface, but in which the organisms achieve a favored position to influence epithelial cell function by the production and export of toxins. These ETEC strains are responsible for a majority of cases of traveler's diarrhea among adults, but they
have also been of importance in infantile or childhood diarrhea in developing countries. In the second type of association, in which adherence of bacteria to host cell membranes is extremely close, there is morphologic evidence that the adherent organisms are associated with epithelial cell damage. This type of association has been increasingly documented for the human EPEC strains that have been associated with outbreaks of infantile diarrhea since the 1950s. A third type of association is exemplified by the EIEC, whose initial association with the intestinal epithelium has not been well studied or documented, but which ultimately invade intestinal epithelial cells in a manner completely analogous to Shigella strains. The EIEC strains are least commonly recognized, but the methods for their recognition are also difficult and generally least available.

For each of these three types of association, we shall review the current knowledge of morphology, of the bacterial determinants, and of the host determinants of bacterial interactions with host cells. Much of this current knowledge of mechanisms derives from studies of animal models of E. coli enteric disease. Brief speculation as to the possible role of these mechanisms in intractable infant diarrhea will follow.

**ETEC ADHERENCE**

**Morphology of ETEC Adherence**

Attachment to, and colonization of, the intestinal mucosal surface without damage to the underlying epithelial cell population is characteristic of the ETEC strains (Fig. 2). It is also characteristic of Vibrio cholerae infection. During infection, these organisms are localized at the mucosal surface, yet with distances of 30 to 50 nm separating their plasma membranes from the intact brush border plasma membranes of the nearest adjacent intestinal epithelial cells (2,5,6). On electron microscopy, the organisms appear to have penetrated the mucus layer and to be lodged or anchored in the glycocalyx of the epithelial cell. The glycocalyx is not seen on standard transmission electron microscopy, but its presence can best be demonstrated with special stains such as ruthenium red if care is taken to stabilize it during EM fixation to prevent its collapse on dehydration (2). Since these toxigenic organisms produce net intestinal secretion of water and electrolytes (diarrhea) by secreting specific well-characterized enterotoxins (cholera toxin, the analogous E. coli heat-labile toxin, and the unrelated E. coli heat-stable toxin) that interact with the intestinal epithelial cells, their observed position appears entirely appropriate to the primary mechanism of diarrhea as presently understood.

**Bacterial Determinants of ETEC Adherence**

In the case of the enterotoxigenic E. coli, the best established determinants of adherence (adhesins) have been shown to be specific types of nonflagellar,
FIG. 2. Schematic representation of the stages of mucosal adherence and surface colonization by enterotoxigenic E. coli (ETEC). ETEC organisms adhere to the mucosal surface (A) and colonize it (B) without apparent damage to microvilli of the underlying absorptive epithelial cells. Adherence is mediated (C) by specific interactions between bacterial adhesins and complementary receptors on the host mucosal surface. Pili have been identified as the bacterial adhesins for most ETEC strains.

filamentous, proteinaceous appendages termed pili by Brinton (16) or fimbriae by Duguid (17). Smith and Linggood (18) provided the classical evidence for the role of these surface antigens in intestinal colonization when they showed that the K88 antigens previously described by Orskov and Orskov (19) conferred on enterotoxigenic E. coli the ability to colonize the small intestine of piglets and to produce diarrhea. These studies were possible since the K88 antigen (which proved not to be acidic polysaccharides, as the K designation implies, but fine filamentous pili) and the E. coli ST are each encoded by separate plasmids. This permitted the preparation of strains that produced only K88 (K88+, ENT−), only enterotoxin (K88−, ENT+), both (K88+, ENT+), or neither (K88−, ENT−) for use in animal challenge studies. Only the K88+, ENT+ strain produced diarrheal disease comparable to the natural infection; K88−, ENT+ strains had no effect; K88+, ENT− strains colonized the intestines and also produced a degree of diarrheal disease. This has led to subsequent speculation about the possibility of a separate role for adherence factors in intestinal fluid secretion independent of enterotoxin.
Other adherence pili have subsequently been described and have been variously designated as K antigens (20), colonization factor antigens (CFA) by Evans et al. (4,14), and F (fimbrial) antigens by Morris et al. (21). Like other pili, these are helical rods with diameters from 2 to 7 nm, which are composed of repeating polypeptide subunits (MW range from 3,000 to 30,000). These hair-like structures reach lengths of several hundred nanometers and estimated molecular weights of several megadaltons. In some cases, their immunological differences have been confirmed by amino acid composition and sequence studies. A partial list, in addition to K88, would include, K99 (20), 987P (22), and F41 (21), which confer adherence in calves and sheep, and CFA/I (4) and CFA/II (23), which mediate colonization of the human small intestine. Others remain to be documented (23).

Genetic information for all of the enteroadherence pili described to date resides on transmissible plasmids. Smyth et al. (24) and Wadstrom et al. (25) have demonstrated in vitro that piliated enteroadherent ETEC organisms have pronounced hydrophobic binding characteristics using hydrophobic interaction chromatography. These characteristics distinguish the enteroadherence pili of ETEC from the more hydrophilic type 1 pili. All of these enteroadhesins are immunologically and, if careful observations are made, morphologically distinguishable from each other and from the type 1, or common, pili, which can be produced by most E. coli strains given the appropriate growth conditions. Although type 1 pili do have adhesive properties, most notably for guinea pig erythrocytes, which they hemagglutinate in a D-mannose-sensitive fashion, type 1 pili have not been shown to have a primary role in adherence in the gastrointestinal tract.

In vitro observations have demonstrated that expression of enteroadherence pili can be regulated by growth conditions. None of these adhesins are produced at 18°C; they are best produced at 37°C. In general, pilus production is best in minimal media, and complex or enriched media tend to suppress pilus expression. Specific suppressive effects have been observed for both glucose and amino acids. In addition, some pili are better expressed at the surface of agar plates than in broth. Once pilus production begins under a given set of growth conditions, daughter organisms tend to remain in the piliated phase through a control mechanism at the gene level that does not involve a gain or loss of genetic information. This phenomenon has been termed phase variation (16). How these in vitro conditions and observations are mimicked in the in vivo situation is unknown, and this raises questions about the in vivo expression of adhesins. Nagy et al. (22) have provided data supporting the selection of piliated forms in vivo based on colonial morphology. Although the accumulated evidence for pili as major determinants of host-specific intestinal adherence is compelling, morphological documentation of the presence of pili on attached organisms in vivo has been difficult and remains incomplete in most cases. Recently, Chan et al. (2) provided evidence that the fine strands seen tethering enterotoxigenic
enterocyte adherence of escherichia coli

organisms to the mucosa of the calf are immunologically identifiable as K99 pili.

With the ETEC strains, a well-developed bacterial capsule (slime layer glycocalyx) can be demonstrated on electron microscopy if care is taken during fixation to prevent dehydration and to stabilize the structure with specific antibody. This structure surrounds each of the adherent organisms, permitting the organisms to interact with each other, thereby forming interadherent microcolonies or “consortia” of organisms in Costerton’s terminology (26). Only a portion of the adherent encapsulated organisms appears to be positioned appropriately to interact directly with host epithelial components. Thus, the bacterial capsule appears most important in permitting aggregation of bacteria, whereas the attachment to some part of the mucosa is mediated by pili, which may protrude through the capsule.

Host Determinants of ETEC Adherence

Interactions of adherence pili with the intestinal mucosal surface appear to have many of the characteristics of specific adhesin/receptor interactions, which are analogous to the interaction of plant lectins with binding sites on mammalian cells. Binding of piliated organisms to erythrocytes has been used as an in vitro model of E. coli adherence to host cells. Red cell binding of piliated organisms may not involve the same interactions as those with intestinal cell membranes. Tissue specificity of infection and colonization is very likely a result of highly specific molecular interactions. Enteroadherent piliated organisms bind to a variety of red cells, and patterns of binding to red cells of different animal species have been used to classify differences among pili. All enteroadherent pili confer binding that is not inhibited by D-mannose, a characteristic that distinguishes them from type 1 or common pili.

Other studies have utilized isolated epithelial cells, or brush borders from these cells, to characterize binding and the nature of the epithelial receptors. Attempts to inhibit binding in these systems with simple sugars have generally been ineffective, although more complex carbohydrate structures as expressed on native gangliosides may inhibit binding in the cases of K88, K99, and CFA/I. Correlation of in vivo and in vitro data suggests that epithelial receptors for enterotoxigenic E. coli are both genetically determined and age related. Using in vitro assays of binding to brush border membranes, Sellwood et al. (27) have described two pig phenotypes, adhesive and nonadhesive, which, respectively, possess and lack mucosal receptors for the K88 antigen. These phenotypes correlate with susceptibility to infection with K88+ organisms. Thus, genetic differences in epithelial receptors for adhesins may help to explain relative differences in susceptibility of different population groups to infection with particular organisms. Kearns and Gibbons (28) have also suggested that there are differences in glycolipid composition of the brush borders of K88-adhesive and -nonadhesive
piglets. These findings are consistent with the demonstration of specific glycolipids as receptors for adherent *E. coli* in the urinary tract.

Carbohydrate determinants found on membrane glycolipids may also be expressed on glycoproteins in the same cells or tissues. Glycolipids (glycosphingolipids) are present in great abundance in the apical plasma membranes of intestinal epithelial cells, but Slomiany and Slomiany (29) have shown that the more complex glyceroglucolipids are also present in the more superficial layers and in the mucus layer. Thus, the observed association of enterotoxigenic *E. coli* with the intestinal surface may be the result of interaction of adhesive pili with specific complex carbohydrate receptors in the cell membrane (glycolipids or intrinsic glycolipids) or the glycocalyx (extrinsic glycoproteins or secreted glycolipids). Much work remains to be done in characterizing these receptors and their densities in different locations. A balance among receptors on the membrane, in the glycocalyx, and in the mucus layer may determine whether organisms expressing adhesins are swept away in the mucus, remain embedded in the glycocalyx, as appears to be the case for the enteropathogenic *E. coli*, or achieve a more intimate association with the epithelial cell membrane.

**EPEC ADHERENCE**

**Morphology of EPEC Adherence**

Recently, a more intimate type of association (Fig. 3) has been documented between bacterial and mucosal cells in the case of the enteropathogenic *E. coli* strains causing epidemic infantile diarrhea (10–12). A small group of *E. coli* serotypes associated with outbreaks of diarrhea in young infants, frequently in a nursery setting, have been recognized since the 1950s. Over the last decade, interest in these serotypes had waned, largely because of the inability to demonstrate the production of classical *E. coli* enterotoxins by the organisms. However, the importance of the serotypes has been reaffirmed by their continued appearance in association with disease and by the demonstration of their ability to cause disease in challenge studies in human volunteers by Levine et al. (30).

In 1980, Ulshen and Rollo (31) demonstrated, on intestinal biopsy, a close association of *EPEC* organisms (strain 0125ac:H11) with the duodenal mucosal surface of an infant with chronic diarrhea. This association was clearly distinguishable from that described for the enterotoxigenic strains. The organisms were closely associated with the apical surface of the epithelial cells, with only 10 to 12 nm separating the apical plasma membrane from the bacterial cell membrane. Characteristic microvilli are absent in the area of adherence; there is a disruption or absence of the normal cytoskeletal elements that make up the microvillus core. The apical plasma membrane partially surrounds the attached organisms in a cup-like or pedestal-like extension. Although the appearance is suggestive of an early stage of receptor-mediated endocytosis, no organisms are actually invading the epithelial cells.
FIG. 3. Schematic representation of the stages of mucosal adherence and surface colonization by enteropathogenic *E. coli* (EPEC). EPEC organisms initially adhere to the intact mucosal surface (A) without damage to the microvilli. As colonization proceeds, a late stage of adherence occurs (B) in which there is loss of apical microvilli associated with disruption of their cytoskeletal structures. The mechanism of initial adherence (C), at least in the case of the rabbit strain RDEC-1, appears to be similar to that of ETEC. The mediators of the late stage of EPEC adherence (D), in which organisms rest on pedestal-like extensions of intact plasma membrane, are unknown.

This characteristic appearance has been documented consistently in subsequent established outbreaks of EPEC diarrhea by other observers such as Rothbaum et al. (11) and Clausen and Christie (10). It now seems that this type of close enteroadherence is characteristic of the EPEC strains. The bacterial and host determinants of this interaction remain unknown, although some type of adhesin/receptor interaction is suggested. To date, only type 1 pilus production has been demonstrated by the EPEC strains, but, as previously pointed out, this is a common capability of *E. coli* and does not distinguish the EPEC strains from many nonpathogens; thus, type 1 pili are unlikely to be responsible. Adherence of EPEC strains to human intestinal tissue *in vitro* has not been reported, although several authors have described the adherence of these organisms to tissue culture cell lines. Several communications of the association of large plasmids with *in vitro* association of EPEC strains in tissue culture have been presented or published in preliminary form.
Khavkin et al. (32) provided some insight into the events leading up to close EPEC adherence. These authors, using fluorescent PAS staining with fluorimetry to give a quantitative measurement of the thickness of the mucosal polysaccharide layer (glycocalyx), showed marked thinning of this layer over the brush border during infection with the enteroadherent EPEC strain 026:K60:H11 (a strain that also produces Vero cell toxin). In contrast, the ETEC strain B7A (0148:H28) caused only localized changes of the brush border polysaccharide in the areas closely adjacent to the adherent organisms. In the case of ETEC, the minimal thinning of the polysaccharide layer seemed to follow adherence. The changes in the polysaccharide layer in the case of the EPEC strain could be reproduced by perfusion of the loop with culture filtrates presumably containing toxins. These studies suggest that dissolution of the peripheral polysaccharide coat of the brush border, perhaps induced by toxins, may precede close membrane adherence of EPEC strains.

Morphologically, the picture of adherence seen with the EPEC strains to the intestinal mucosa of human infants is indistinguishable from the adherence of the RDEC-1 strain of E. coli (Fig. 4) to rabbit intestine as shown by Takeuchi

![Figure 4](image-url)

**FIG. 4.** High-voltage electron micrograph of a 0.5-nm section of cecal mucosa from a rabbit infected with RDEC-1 strain of Escherichia coli illustrating early and late stages of RDEC-1 adherence. Several bacteria (B) are seen adhering to mucosal epithelial cells (MEC) and sloughed mucosal epithelial cells (SMEC). Early adherence to intact microvilli (MV) is seen as well as late stage adherence (arrow) with pedestal (P) formation. The tissues were treated with antisera to RDEC-1 OK antigen, and the inset demonstrates pilus-like structure (arrow) protruding through the bacterial glycocalyx. (From Cantey et al., ref. 9, with permission. Illustration courtesy of Dr. J. R. Cantey).
et al. (12) and by Cantey et al. (9). RDEC-1 is an 015:K?:NM E. coli that causes an endemic diarrheal disease in young weaned rabbits (8). After inoculation with as few as 100 organisms, colonization of the ileum, cecum, and colon of the rabbit occurs within 5 days. Diarrheal disease ensues in 80% of animals within 7 to 10 days, and there is a mortality of approximately 40%. Diarrhea is accompanied by dense adherence of the organisms to the mucosal surface of the cecum and lesser, but definite, adherence of the organisms to the tips of the ileal villi. On electron microscopic examination, adherent organisms are closely apposed to the apical membrane of ileal absorptive cells, which have lost their characteristic microvilli. Pedestal formation and microvillus core disruption are seen as in the case of the human EPEC organisms (9). This is interpreted as a late-stage adherence. In addition to this characteristic close or late association, Cantey has also shown that some RDEC-1 organisms remain more peripherally attached in the region of the epithelial glycocalyx in areas where microvilli remain intact (9). This is very similar to the picture of ETEC adherence and appears to be an early stage in the adherence process.

It is possible that there are separate bacterial and host determinants for these early and late stages of adherence to absorptive epithelial cells. Very recently, Inman and Cantey (33) described a very early interaction of RDEC-1 with the M cells overlying Peyer's patches in the ileum. This occurs within 2 days of inoculation, at a time when adherence to absorptive cells is not yet seen. The association with M cells also appears to have its peripheral and close components. Since these M cells presumably process antigenic information and transmit it to underlying gut-associated lymphoid cells to regulate local mucosal immune responses, this interaction may be of great importance in the host's response to infection. It remains unknown whether the host and bacterial determinants of these interactions of RDEC-1 with the immune system (M cells) are related to those mediating interactions of RDEC-1 with absorptive cells.

Bacterial Determinants of EPEC Adhesion

In elegant high-voltage electron micrographs with specific antibody staining for the capsular polysaccharide of RDEC-1, Cantey (9) has shown that bacterial surface polysaccharide is present during the early stage of in vivo interaction of RDEC-1 with the rabbit intestine and that surface polysaccharide can be stained in the 10- to 12-nm space between closely adherent bacteria and the apical membrane. Although these studies demonstrate the presence of polysaccharide during in vivo adherence of RDEC-1, they do not indicate that this substance is necessary for adherence. Studies in our laboratory (34) have demonstrated in vitro adherence of RDEC-1 E. coli to isolated rabbit intestinal brush border membranes (Fig. 5A). This in vitro process is species specific in that RDEC-1 does not adhere to similarly prepared brush borders from rat, guinea pig, or human ileum. Additional studies have shown that RDEC-1 does not infect these species (35). Adherence to rabbit ileal brush borders is approximately
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FIG. 5. In vitro interactions of RDEC-1 with isolated rabbit ileal brush borders. A shows a phase-contrast photomicrograph of piliated RDEC-1 organisms adhering to ileal brush borders. The inset is an electron micrograph of the negatively stained surface of one organism expressing numerous pili. B demonstrates the failure of nonpiliated RDEC-1 to interact with the same rabbit brush borders. Pili expression was suppressed by growth of the organisms in enriched medium. The inset is an electron micrograph of the negatively stained surface of a typical organism lacking pili. The pili, which determine RDEC-1 adherence to rabbit ileal brush border, are designated AF/R1.

twice that seen to jejunal brush borders, a finding that fits with the distal enteroadherence seen with the disease. The in vitro process is related to the expression by the RDEC-1 organisms of specific adherence pili (36,37).

Three types of evidence have been developed to show the dependence of in vitro RDEC-1 adherence on specific pili termed AF/R1, which are distinct from type 1 pili and from other classes of adherence pili described to date. First, the expression (Fig. 5B) of RDEC-1 pili can be suppressed by growth in enriched media. Loss of pilus expression correlates with loss of in vitro adhesiveness (37). Second, RDEC-1 pili were transferred to nonpiliated Shigella strains. Transfer of piliation, which correlated with the transfer of an 85-megadalton plasmid, correlated with transfer of in vitro adherence to brush borders (37). Finally, RDEC-1 pili were isolated and shown to adhere to the mucosal surface of rabbit ileum in a species-specific manner and with a distribution comparable to that seen for the whole organism (36).

RDEC-1 pili might mediate the early or late stage of RDEC -1 adherence seen in vivo. We favor the interpretation that RDEC-1 pili mediate the stage
of close attachment to the membranes of absorptive epithelial cells. This is based on our observation that preparations of apical plasma membranes from rabbit ileum, which are essentially free of glycocalyx, interact readily with piliated RDEC-1 \textit{in vitro}. When this interaction is observed by electron microscopy, it resembles the close association seen \textit{in vivo} in that there is only 10 to 15 nm separating the rabbit apical plasma membranes from the bacterial cell membrane (P. A. Schad, C. P. Cheney, and E. C. Boedeker, \textit{unpublished observations}). On the other hand, observations on the development of brush border receptor activity have shown that adherence develops in parallel with the glycocalyx (38). Whatever the site of pilus-mediated RDEC-1 adherence, the preceding observations are of importance, since, if the analogy between RDEC-1 and \textit{EPEC} infection is correct, new adherence pili may be found on the \textit{EPEC} strains. Perhaps these pili are only to be expressed under certain growth conditions, which may obtain in the intestinal tracts of susceptible infants.

\section*{Host Determinants of \textit{EPEC} Adhesion}

Children infected with enteroadherent \textit{EPEC} strains in the study of Rothbaum et al. (11) ranged in age from 3 to 24 weeks and tended not to have been breast fed. No conclusive information is available to suggest that infected infants have underlying or preexistent disease, although many of the infants had been previously hospitalized. For RDEC-1 infection, previously healthy animals can be readily infected, suggesting that no preceding mucosal injury is required.

Using the RDEC-1 model, we have studied the development of the \textit{in vitro} association between RDEC-1 and intestinal brush borders (38). Receptor activity for ileal brush borders cannot be detected before day 21 but reaches adult levels by day 28 to 35. Thus, the development of receptor activity correlates with the marked changes in the intestinal epithelium seen at weaning, which include a shift from lactase to sucrase and isomaltase activity on the brush border, a loss of immature vacuolated cells, and the repopulation of the villus tips with mature enterocytes. These findings suggest that susceptibility to infection seen at weaning may be related not only to a loss of passive maternal antibody protection but also to the development of new receptor activity specific for enteropathogens. These \textit{in vitro} studies have been corroborated by recent \textit{in vivo} studies in which ileal adherence was never seen before day 20, although receptor activity in the cecum appears to develop somewhat earlier than in ileum (between days 16 and 20).

Receptor activity (measurable as bacterial agglutinating activity for piliated RDEC-1) has been solubilized from adult rabbit ileal brush borders using detergents. This soluble receptor activity appears to reside in a glycoprotein fraction of the brush borders (C. P. Cheney and E. C. Boedeker, \textit{unpublished observations}). These findings are in contrast to the previously mentioned evidence that \textit{ETEC} receptors are expressed on glycolipids.

The mechanisms whereby \textit{EPEC} strains and RDEC-1 induce epithelial damage
and intestinal secretion are unknown. By analogy to EPEC strains, the favored hypothesis is the secretion of an enterotoxin or toxins. The 026:K60:H11 enteroadherent EPEC strain has been associated with a toxin for Vero cells in tissue culture (39), but this is not a common property of EPEC strains and may serve only to set this strain apart from the rest.

Very recently, O'Brien et al. (40) have confirmed their earlier observation that RDEC-1 produces a shiga-like toxin with cytotoxic and enterotoxic effects. Furthermore, they have demonstrated that this capacity for toxin production is shared by a large number of E. coli strains including both EPEC(0142K16:H6, 0128K67,H2) and ETEC (including H10407 serotype 078:K11:H2) strains. These organisms produce low to moderate levels of shiga-toxin-like material when compared to the levels produced by S. dysenteriae. Such a toxin may primarily be involved in depletion of mucus and glycocalyx layers, thereby permitting membrane adhesion, as suggested by the work of Khavkin et al. (32). Alternatively, the toxin may be primarily involved in cellular damage, a capability suggested by its ability to inhibit protein synthesis. Since toxin production has been demonstrated for such a wide variety of strains of E. coli, it is possible that adhesive factors permitting extremely close apposition of the toxin-producing strains to cell membranes may also be required for the strains secreting low levels of toxins to exert their pathogenic effects.

Another hypothesis that deserves consideration is that the cellular damage and cytoskeletal disruption seen with the EPEC strains may be induced by the bacterial adhesins. These molecules may have sufficient membrane-active properties, conferred by their own hydrophobic segments or their lectin-like ability to aggregate intrinsic membrane proteins, to change membrane permeability. Changes in brush border membrane permeability to calcium ion, for example, could induce the apical cytoskeletal disruption characteristic of EPEC infection.

**EIEC MUCOSAL ASSOCIATION**

The EIEC strains are least commonly recognized in infantile diarrheas (41), and in comparison to the ETEC and EPEC, relatively little is known of the determinants of their interaction with the intestinal mucosa. These strains are genetically closely related to Shigella strains, whose activity they mimic. Like Shigella, they induce an early disruption of microvillus membranes with actual loss of continuity of these apical membranes (14). This is in contrast to the EPEC strains, which disrupt cytoskeletal structures in the apical cytoplasm but leave the apical membrane intact. Shortly after disruption of the apical membranes, the organisms are found within the cytoplasm of epithelial cells enclosed in vacuoles, where they are capable of multiplying. As with Shigella, they have been most extensively studied in the starved, opiated guinea pig, although studies in rabbits, monkeys, and human volunteers have been performed (42). In human volunteer studies, challenge doses of $10^5$ to $10^6$ have been required, in distinction to the ability of $10^1$ or $10^2$ Shigella to induce disease in humans, indicating
differences in virulence between Shigella and EIEC. Pathogenic factors of either Shigella or EIEC may be divided into several determinants, which include the ability to interact with host membranes, the ability to invade or be taken up into the cell, and the ability to multiply in the cell. Most of these virulence properties are chromosomally determined in Shigella, although recently, in Shigella, evidence for virulence determinants on large plasmids has been described (43). The standard test for invasiveness is the development of keratoconjunctivitis in the guinea pig eye, as described by Sereny (44). Attachment to HeLa cells in vitro in tissue culture and uptake into these cells have been used to supplement the Sereny test. In Shigella, plasmid-coded outer membrane proteins appear to be determinants of HeLa cell attachment and uptake (43).

Recently, Cantey et al. (13,15) described a model of enteroinvasive E. coli using strain 0143:K?:H-, which involved no special treatment of the animals to induce disease. Of interest, Cantey noted a dissociation between activity in the Sereny test and the ability to invade the rabbit intestine. Strains remained Sereny positive over long periods but gradually lost their ability to invade the rabbit intestine on passage. Invasive ability for rabbit intestine could be restored by instilling the organisms into the guinea pig eye. This information is in contradiction to earlier evidence that suggested that the Sereny test might be insensitive in screening stored EIEC strains. In this model, Cantey was never able to observe an initial stage of association with the cell surface, and he suggested that penetration took place by membrane lysis. Adherence pili have not been described in these strains or in Shigella.

Izhar et al. (45) have recently described a lectin-like receptor (mucosal adhesin) for nonpiliated Shigella strains loosely associated with guinea pig colonic cells. Adherence mediated by this factor was inhibitable by fucose or glucose. Since the factor could be washed off the cells, it is suggested that the factor may reside in the mucus or peripheral glycocalyx of the host. The soluble factor could agglutinate EIEC but not EPEC strains. One interpretation of these data is that the guinea pig colon is normally protected from invasion by a soluble lectin in the mucus. Only when this factor is depleted, as in starvation or opiate treatment, does the guinea pig colon become susceptible to invasion by Shigella-like organisms. If this interpretation is correct, naturally susceptible hosts for EIEC and Shigella, such as humans and primates, may be deficient in such protective factors.

SUMMARY

Mechanisms for the interaction of three broad classes of pathogenic E. coli with the intestinal epithelium have been reviewed. In the development of intractable infantile diarrhea, the mechanisms recently described for EPEC adherence seem the most relevant, since they provide for close mucosal colonization with concomitant epithelial cell damage. This damage may leave the mucosal cells compromised for a time, even after bacterial colonization has been elim-
inated by antibiotic therapy. The determinants of adhesiveness of EPEC strains infecting humans remain to be elucidated, although the model of RDEC-1 infection of rabbits provides clues as to the types of interactions involved. In particular, the age-related development of mucosal receptors for RDEC-1 at the time of weaning, when mucosal immunity is just developing, and when tolerance may result from interactions of EPEC antigens with the immune system, suggests reasons for particular susceptibility to these infections in infants. Our developing understanding of these mechanisms may suggest measures for preventing these associations, as is discussed elsewhere in this volume (E. C. Boedeker, this volume).

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REFERENCES

36. Boedeker EC, Cheney CP. Pili as adherence factors in Escherichia coli strain RDEC-1. In:

37. Cheney CP, Formal SB, Schad PA, Boedeker EC. Genetic transfer of a mucosal adherence factor (R1) from an enteropathogenic Escherichia coli strain into a Shigella flexneri strain and the phenotypic suppression of this adherence factor. J Infect Dis 1983;147:711-23.


