REVIEW

Virulence factors of enteropathogenic *Escherichia coli*

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Introduction

Escherichia coli is part of the normal large-bowel flora of man and animals. Although most strains of *E. coli* are non-pathogenic in the intestine, some can produce diarrhoea and by a number of distinct mechanisms.

E. coli was first incriminated as an enteropathogen when Bray (1945) demonstrated that an antigenically distinct strain of *E. coli* was responsible for an outbreak of infantile diarrhoea. These strains were named enteropathogenic *E. coli* (EPEC) by Neter *et al.* (1955). Studies in many locations showed that EPEC strains were isolated with greater frequency from infants with diarrhoea than from healthy infants. Identification of EPEC strains isolated from faecal samples was by O-serotype determination since virulence mechanisms could not be detected in these strains (Levine and Edelman, 1984).

In the 1970s it was shown that some strains of *E. coli* produced a heat-labile (LT) or a heat-stable (ST) toxin or both, or had entero-invasive properties. These strains were classed enterotoxigenic (ETEC) and entero-invasive (EIEC) *E. coli*, respectively (Rowe, 1979). Examination of EPEC strains for the above described virulence traits yielded in the main negative results (Goldschmidt and Dupont, 1976; Gross *et al.*, 1976; Levine *et al.*, 1978). Some workers believed that the lack of identifiable virulence properties in EPEC strains justified the abandonment of their serotyping (Sack, 1976); others suggested that mechanisms distinct from those described might exist in EPEC strains and that serotyping should be continued (Rowe *et al.*, 1975). This controversy was to some extent resolved when Levine *et al.* (1978) showed that EPEC strains negative in tests for LT, ST and invasiveness were, nevertheless, able to induce diarrhoea in human volunteers. This observation provoked intensive study of the virulence characteristics of *E. coli* and it is now apparent that strains can produce distinct intestinal disease in man by several different

mechanisms. The main features of these strains of *E. coli* are shown in the table.

The function of this review is to describe in detail the virulence attributes of enteropathogenic strains of *E. coli* and their relationship with the virulence factors of other strains of *E. coli* and other enteric pathogens.

Enterotoxins

Animal studies. Early studies of enterotoxic activity in EPEC strains used the rabbit ileal-loop (RIL) model, a technique widely used for detecting enterotoxins and enterotoxigenic organisms that induce dilation of the ileal loop and cause fluid secretion into the lumen (De and Chatterjee, 1953). De *et al.* (1956) observed that three strains of EPEC could induce fluid accumulation and dilation of the RIL. Taylor *et al.* (1958) similarly detected enterotoxic activity in an EPEC strain of serogroup O26 and showed that ileal loops varied in their reactivity with the outcome being influenced by the strain of rabbit, diet and the bacterial growth medium.

Among EPEC strains of various serotypes isolated from children with and without diarrhoea, Taylor *et al.* (1961) showed that only strains isolated from symptomatic infants induced fluid secretion in the RIL. A labile material isolated from those strains was absent, however, from strains of similar antigenic structure isolated from other sources. This labile material was thought to be responsible for producing diarrhoea.

Sakazaki *et al.* (1974) examined 40 EPEC strains of which 27 caused dilation in the RIL. For any positive strain, however it was not uncommon to obtain only one of two or three loops positive. With some strains, culture filtrates gave better results than live cultures and *vice versa*. A rough variant of a strain able to produce dilation in the RIL was shown to be negative in this system. Culture filtrates of both smooth and rough strains could induce dilation suggesting a role for the O antigen in the production of fluid secretion by live organisms.

In contrast to the results described by Sakazaki

Table. Properties of *E. coli* strains

Category	Serogrouping	Virulence factors
Enterotoxigenic <i>E. coli</i> (ETEC)	O6, O8, O15, O20, O25, O27, O63, O78, O80, O85, O115, O128ac, O139, O148, O153, O159, O167	Heat-labile (LT) and heat-stable (ST) toxins; adhesive factors CFAI, CFaII and E8775
Entero-invasive <i>E. coli</i> (EIEC)	O28ac, O29, O124, O136, O143, O144, O152, O164, O167	Epithelial invasion; Shiga toxin
Enterohaemorrhagic <i>E. coli</i> (EHEC)	O157, O26, O111	High levels of Shiga toxin; fimbrial adhesin
Entero-adherent <i>E. coli</i> (EAEC)	?	Epithelial adherence
Enteropathogenic <i>E. coli</i> (EPEC)	O18ac, O26, O44, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142	Shiga toxin production; fimbrial or outer-membrane protein adhesin in some strains

et al. (1974), Zinkernagel and Colombini (1975) found that various EPEC strains could cause fluid secretion but they were unable to detect enterotoxins in whole-cell lysates or culture supernates. It was suggested that poor production of toxin *in vitro* might account for this finding. A further study with the RIL model showed that only 3 of 48 EPEC strains were reactive (Goldschmidt and DuPont, 1976). Two of these were of serogroup O26, the other belonged to serogroup O111. Similar findings were obtained by Polotsky *et al.* (1977) who, with live cultures, showed that only two strains of serogroup O26 gave a constant response; some strains of serogroup O111 showed variable results, whereas strains of other serotypes gave negative results. Gorbach and Khurana (1972) found that an infant RIL was more sensitive than an adult RIL for detecting enterotoxigenic activity in EPEC strains and, with this model, demonstrated enterotoxigenic activity in EPEC strains of various serotypes.

Other workers, unable to obtain reactions with EPEC strains in the RIL model, obtained better results with infant mouse ileal loops, but again animal-to-animal variation was also found (Punyashtiti and Finkelstein, 1971). With the more sensitive rabbit-skin permeability-factor (PF) test, in which toxins induce distinct skin lesions, Evans *et al.* (1973) detected enterotoxigenic activity in EPEC strains. The activity differed quantitatively and qualitatively from that of similar preparations from ETEC strains and again false-negative results were not uncommon even when concentrated culture supernates of EPEC strains were tested. Strains of proven pathogenicity have been shown to be non-enterotoxigenic in the PF test (Robins-Browne *et al.*, 1982) and the infant RIL model, although

reactivity was found in a canine-loop system (Levine *et al.*, 1978).

Klipstein *et al.* (1978), using a perfused rat-jejunum assay, showed that a number of EPEC strains were able to elaborate potent heat-labile and heat-stable enterotoxins which induced fluid secretion in this system. These toxins were distinct from LT and ST of ETEC strains and there was a correlation in this assay between the potency of the toxin and the severity of the disease caused by a particular strain of EPEC.

Although the results from these studies are often contradictory, they do suggest on balance that some form of enterotoxigenic product is elaborated by EPEC strains and that it is distinct from both LT and ST. The models which have been used, however, appear to lack sensitivity and show marked animal-to-animal variation, which may explain in part the lack of consistency of results. It is also likely that there is variation in the yields of toxin from different EPEC strains. Cultural conditions and different preparation methods may also affect the potency of enterotoxin preparations. It is apparent, however, that amongst EPEC strains, those belonging to serogroup O26 and, to a lesser extent, serogroup O111 produce higher yields of toxin, or are more likely to produce toxin, than strains of other serotypes.

Tissue-culture methods. Because the heat-labile toxin (LT) of *E. coli* and cholera toxin (CT) itself were found to produce distinct morphological changes on various tissue-culture cells, e.g., Chinese hamster ovary cells (CHO) (Guerrant *et al.*, 1974), these methods were applied to EPEC strains. Numerous studies showed, however, that the majority of EPEC strains did not produce these

toxins (Goldschmidt and Dupont, 1976; Gross *et al.*, 1976; Levine *et al.*, 1978) and Robins-Browne *et al.* (1982) using gene probes to detect nucleotide sequences for LT and ST, showed that EPEC strains did not have the genetic information to code for these toxins.

Adenylate cyclase-stimulating activity. The elucidation of the mode of action of *E. coli* LT and CT as stimulators of membrane adenylate cyclase (AC) prompted investigation of EPEC strains for toxins with a similar mode of action. Scheftel *et al.* (1980) detected a protein in culture supernates of EPEC strains that could stimulate the AC activity of rat-heart membranes in a manner similar to CT. The AC-stimulating factor was isolated from an EPEC strain of serogroup O111 and found to be a heat-labile protein the mol. wt of which was 30×10^3 ; some preparations, however, were complexed with polysaccharide and were heat-stable. The toxin stimulated AC and induced fluid secretion in the RIL though to a lesser extent than CT. A similar toxin isolated from an EPEC strain of serotype O126 stimulated AC activity in infant-rabbit intestinal tissue but not adult tissue (Kantor *et al.*, 1974). Long-Krug *et al.* (1984) examined EPEC strains and showed that none produced an AC-stimulating factor and Law *et al.* (1987), using strains known to be pathogenic in volunteers, showed that culturing these strains in conditions known to promote enterotoxin production by other pathogens did not lead to the detection of an AC-stimulating factor in those strains.

The conflicting results from these studies may be due to differences in the assay conditions employed. The ATP concentration is particularly important, and AC-stimulating activity was detected only in the assays in which high concentrations of ATP were employed. CT and LT can bring about adenylate-cyclase stimulation when the ATP concentration is low, although some toxins, e.g., Shiga toxin, can activate AC when the ATP concentration is high (Charney *et al.*, 1976). Such a toxin may be produced by EPEC strains but consensus of opinion is that EPEC strains do not elaborate an LT-like toxin.

Cytotoxins

As well as examining EPEC strains for toxins that can induce fluid secretion from the intestine, many workers have also examined these strains for cytotoxins, i.e., toxins that damage and kill cells.

Vero cytotoxin. In 1977, a cytotoxin shown to be produced by some strains of *E. coli*, notably of serogroup O26 (Konowalchuk *et al.*, 1977), was

detected by its cytotoxic effect on Vero tissue-culture cells, was distinct from LT and was called Vero toxin (VT). It did not produce any change on CHO or Y1 adrenal cells and little response with RIL. A further study showed that VT had a mol. wt of 28×10^3 , that it produced mild fluid secretion in the RIL but did not stimulate AC (Konowalchuk *et al.*, 1978).

Examination of 252 strains of *E. coli* for VT found that only three strains gave positive results; these belonged to serotype O26, but another five strains of that serotype gave negative results. Removal of plasmids from a toxigenic strain did not affect toxin production (Wade *et al.*, 1979) and Scotland *et al.* (1983b) found that VT genes were carried on bacteriophage in one of their strains. In another study of 253 EPEC strains from 11 different O-serogroups, only 25 produced VT and of these, 23 belonged to serotype O26 (Scotland *et al.*, 1980). The same study suggested that although VT might have a role in the pathogenesis of EPEC diarrhoea, other factors were likely to be involved because some VT-negative strains were known to be pathogenic in human volunteers. Wade *et al.* (1979) also presented evidence of the possible significance of VT when they showed that all three VT-positive strains of serogroup O26 in their study were associated with bloody diarrhoea; the diarrhoea caused by EPEC strains is not usually bloody.

It seems likely that, in previous animal studies, the toxic activity detected in EPEC strains was due to VT because of the strong association of positive reactions in the RIL by strains of serogroup O26 that also produce VT.

In 1982 strains of *E. coli* belonging to serogroup O157, a type not previously recognised as pathogenic, were implicated in outbreaks of haemorrhagic colitis in the USA (Riley *et al.*, 1983). Although these organisms were found to be non-invasive and produced neither LT nor ST, they elaborated large quantities of VT (Johnson *et al.*, 1983; O'Brien *et al.*, 1983); strains of *E. coli* of O157 were also implicated in cases of haemolytic-uraemic syndrome (Karmali *et al.*, 1983b). It was proposed that haemorrhagic colitis and haemolytic-uraemic syndrome were different manifestations of the same deleterious effect of VT (Karmali *et al.*, 1983a).

Strains of *E. coli* elaborating large amounts of VT and implicated as a cause of haemorrhagic colitis have been termed enterohaemorrhagic *E. coli* (EHEC) (Levine and Edelman, 1984) and Levine (1987) has suggested that EPEC strains of serogroup O26 be classed as EHEC because they resemble EHEC strains rather than EPEC strains in that they produce large quantities of VT and

possess a similar plasmid of mol. wt 60×10^6 that encodes a distinct adhesin.

Shigella dysenteriae serotype 1-like toxin. O'Brien *et al.* (1982) observed that EPEC strains elaborated a toxin similar to the cytotoxin of *Sh. dysenteriae* serotype 1 (SDT) when grown in iron-depleted media. Some strains produced large quantities of this toxin, particularly those of serogroup O26. Other EPEC strains produced smaller amounts including strains previously used in feeding studies; non-pathogenic strains of *E. coli* elaborated trace amounts only of toxin. It was later shown that VT and SDT are almost identical (O'Brien *et al.*, 1983) there being minor differences only between the EPEC toxin and that of *Sh. dysenteriae* (O'Brien and LaVeck., 1983). The heat-labile toxic product detected by Klipstein *et al.* (1978) has been shown to be neutralisable with antibody raised against Shiga toxin (Levine and Edelman, 1984).

To ascertain the importance of the toxin in EPEC strains, some large scale studies have been performed, in one of which 79% of EPEC strains produced SDT as compared with only 24% of strains of *E. coli* from healthy individuals (Cleary *et al.*, 1985). Another study revealed that 59% of strains from outbreaks of diarrhoea were SDT producers. Some strains of *E. coli*, however, that gave negative results when cell sonicates were used, gave positive results when French-press lysates were tested (Marques *et al.*, 1986). Whether all EPEC strains produce SDT to some extent, and whether the inability to detect it results from a lack of sensitivity in the assay system, remains to be determined.

A role has been ascribed to this toxin in strains of *E. coli* which produce large quantities of SDT from serogroups O157 and O26, in which it is believed to be responsible for the production of bloody diarrhoea and haemolytic-uraemic syndrome (Karmali *et al.*, 1983a). The toxin is thought to be involved in EPEC disease but conclusive proof of this is lacking. The main doubts arise because of the very small quantities of toxin that are produced (O'Brien *et al.*, 1982). Toxin production *in vivo*, however, may be greater and toxin delivery to the epithelium may be facilitated by the adhesive capabilities of the organism. Further doubt arises because SDT can be detected in non-pathogenic strains of *E. coli* (O'Brien *et al.*, 1982; Cleary *et al.*, 1985) and in bacteria possessing other potent enterotoxins, e.g., *V. cholerae* and ETEC strains (O'Brien *et al.*, 1982; 1984a). The role of SDT in EPEC disease is most likely to be resolved by feeding studies with toxigenic and non-toxigenic derivatives of EPEC strains.

Several groups have shown that two antigenically distinct forms of cytotoxin from EPEC and EHEC strains exist, only one of which is neutralisable with antibody to Shiga toxin (Scotland *et al.*, 1985; Strockbine *et al.*, 1986). Both toxins probably share some common amino-acid sequences because some DNA relatedness has been demonstrated (Scotland *et al.*, 1985). In addition, the toxin genes in both classes of organism have been shown to be carried by lysogenic phages (O'Brien *et al.*, 1984b; Smith *et al.*, 1984; Karch *et al.*, 1987). This observation is of interest because genes for diphtheria toxin are also phage-mediated and production of that toxin is regulated by iron metabolism.

How SDT induces diarrhoea is as yet unclear, although cytotoxic and enterotoxic properties have been demonstrated (Keusch *et al.*, 1981). Some workers report the inability of the toxin to stimulate adenylate cyclase (Flores *et al.*, 1974; Donowitz *et al.*, 1975), whereas others have detected AC stimulation in assays with high substrate (ATP) concentrations (Charney *et al.*, 1976). Whether this is relevant *in vivo* has yet to be determined. In previous studies in which AC-stimulating activity was detected in EPEC strains, it is likely that this was caused by SDT, for high concentrations of ATP were used in those assays.

EPEC adherence

Adherence is an important factor in the initiation of many bacterial infections and in this connection *E. coli* is no exception. Human strains of ETEC produce fimbrial structures called colonisation factors, that bind to receptors on the intestinal epithelium and mediate adhesion. Although EPEC strains lack these structures, they have been investigated for the presence of other adhesins.

Type-1 fimbriae. Evans *et al.* (1979), examining the haemagglutination (HA) reactions of EPEC strains, showed that 42% of them had a distinct HA pattern termed HAIII that was very similar to the pattern given by common type 1-fimbriate bacteria, except that human red-blood cells were not agglutinated. The fimbriae-mediating HAIII are antigenically similar to type-1 fimbriae (Sherman *et al.*, 1985). Type-1 fimbriae have been shown to mediate adherence to intestinal mucosa although strains expressing these fimbriae are variable in this ability. It has been suggested that these differences may be related to the hydrophobicity and charge of surface components of the bacterial cells (Sherman *et al.*, 1985). Not all EPEC strains, however, carry type-1 fimbriae and other structures are likely, therefore, to be involved in the adhesion process.

HEp2 adherence. Cravioto *et al.* (1979) demonstrated that 80% of EPEC strains showed mannose-resistant adherence to HEp2 tissue-culture cells whereas ETEC and normal-flora strains of *E. coli* rarely adhered. Non-adhesive EPEC strains were found more frequently among strains isolated from early outbreaks, a finding that was considered to be caused by loss of a plasmid-mediated adhesin. Scotland *et al.* (1983a) concluded that the adhesin was probably non-fimbrial in nature although its exact form was not known.

A plasmid of mol. wt $50\text{--}70 \times 10^6$, identified in 31 of 32 EPEC strains, was found to code for HEp2 adherence. Transfer of the plasmid, designated pMAR2, to *E. coli* strain K12 carried with it the ability to adhere to HEp2 cells. Deletion of pMAR2 from an EPEC strain, furthermore, resulted in loss of adherence to HEp2 cells and to the intestinal tissue of colostrum-deprived piglets (Baldini *et al.*, 1983). The adhesin coded by this plasmid has been termed EPEC adhesive factor (EAF) (Levine *et al.*, 1985).

Scaletsky *et al.* (1984) studied the adherence of EPEC strains to HeLa cells and noted that adhesion occurred in two distinct ways; localised adherence (LA) in which organisms attached to one or two small areas of the cell surface or diffuse adherence (DA) in which organisms attached to the whole of the cell surface. LA was associated with EPEC strains of serogroups O55, O86, O111, O119, O127, O128 and O142, the serogroups most commonly associated with diarrhoeal disease. EPEC strains belonging to these serogroups have been designated class-I EPEC, suggesting an important role for the plasmid-coded factor EAF. Class-II EPEC strains include some which adhere diffusely to HEp2 cells and others that do not adhere, and serogroups O44 and O114 are involved (Nataro *et al.*, 1985a). The virulence of some class-II organisms has been clearly demonstrated in feeding studies (Levine and Edelman, 1984; Levine *et al.*, 1985).

Various studies have addressed the nature of EAF, the binding of EAF to cellular receptors, and its role in virulence. The latter was convincingly demonstrated when an EPEC strain lacking the plasmid responsible for EAF was found to have considerably reduced virulence for human volunteers (Levine *et al.*, 1985). In that same study it was established that an antibody response was mounted against an outer-membrane protein of mol. wt 94×10^3 encoded by the plasmid and possibly involved in the adhesion of EPEC strains that show LA. In a study of the LA adhesin (Andrade and Santa Rosa, 1986a) neither fimbriae nor capsules were demonstrable on an LA-positive strain. The adhesin

was susceptible to trypsin treatment and was strongly associated with the cell, again suggesting the involvement of an outer-membrane protein in adhesion. Binding of LA-positive strains to HEp2 cells could be inhibited by carbohydrates although there was wide variation in the degree of inhibition by any particular carbohydrate even among strains of EPEC belonging to the same O-serogroup; that suggests structural variations of an adhesin which binds to glycoprotein receptors on the cell surface.

The nature of the EAF receptor has not yet been determined but some studies have provided important information on this subject. Froman *et al.* (1984) revealed that some EPEC strains were able to bind fibronectin, a glycoprotein present on the surface of many cells. Characterisation of one strain established the existence of two classes of bacterial receptor for fibronectin and showed that these differed in their affinity for fibronectin and susceptibility to binding-inhibition by other glycoproteins.

Working with pairs of EPEC strains with and without the pMAR2 plasmid, Wadstrom *et al.* (1986) showed that the plasmid-containing strain bound fibronectin to a greater degree than a plasmid-free strain, suggesting that fibronectin may be a receptor for the EAF of EPEC strains. However, because some fibronectin is bound by strains lacking EAF, the demonstration of two distinct receptors in EPEC strains and the variations in adhesion-inhibition by different carbohydrates suggest that other adhesins or receptors may be involved.

A DNA probe has been developed that detects the pMAR-coded EAF and can be used for the direct detection of EPEC strains in faecal samples. The use of DNA probes has shown that the genes encoding LA and DA are distinct (Nataro *et al.*, 1985b). There are, nevertheless, discrepant findings. Thus, in one study it was reported that all five EPEC strains of serogroup O86 were LA-positive (Scaletsky *et al.*, 1984) whereas in another study none of 19 strains was LA-positive (Nataro *et al.*, 1985b). It is possible that the possession of different adhesins by EPEC strains may vary geographically.

The DA characteristic is likely to be important among EPEC strains which have this property but further work is needed to characterise this adhesin. There is evidence that the adhesin confers hydrophobic properties on DA-positive strains that may assist in the adhesion process (Nataro *et al.*, 1985a).

EPEC adherence *in vivo*. As well as studying EPEC adherence by *in-vitro* systems, adhesion *in vivo* has been examined in studies that used animal models and, more importantly, adhesion to human tissue during actual infections.

Ultrastructural studies of EPEC infection *in vivo* were first performed on gnotobiotic piglets by Staley *et al.* (1969) who demonstrated distinct lesions in which lengthening and disruption of the microvilli and intimate attachment of bacteria to the epithelial cell surface of the intestine occurred. Similar histological findings have also been noted in rabbit intestinal tissue by Polotsky *et al.* (1977) and in colostrum-deprived piglets by Moon *et al.* (1983).

In a study of human infection, biopsies were taken of duodenal and colonic mucosa from an infant with protracted diarrhoea caused by EPEC strains of serogroup O125ac. Examination of the biopsies revealed lesions identical to those already described (Ulshen and Rollo, 1980). Rothbaum *et al.* (1982) investigated an outbreak of chronic protracted diarrhoea caused by EPEC of serogroup O119. Biopsies of jejunal and rectal mucosa again revealed findings similar to those already described. It was also noted that where bacteria adhered to the mucosa, the epithelial membrane protruded so that the bacteria appeared to rest on a pedestal. These strains were found to produce fimbriae associated with HAIII pattern and a capsule. The role of the latter organelle in adhesion is unknown.

Strains of *E. coli* that attach intimately and efface microvilli from intestinal epithelial cells have been named 'attaching-effacing *E. coli*' (AEEC) (Moon *et al.*, 1983) and it has been shown that attaching-and-effacing activity occurs in EPEC strains belonging to classes I and II (Moon *et al.*, 1983, Tzipori *et al.*, 1985). Thus, that adhesion would appear to be a general property of EPEC strains and to be independent of both LA and DA.

The situation has been clarified in a recent study by Knutton *et al.* (1987b) who demonstrated that EPEC adherence is a two-stage mechanism: (i) an initial attachment of bacteria to the intestinal mucosa in a non-intimate fashion is promoted by plasmid-encoded adhesins, e.g., LA and DA; and (ii) effacing of microvilli and intimate EPEC attachment, which may occur in the absence of the first stage, although the plasmid-encoded adhesin enhances the colonising ability of EPEC strains. These workers also presented evidence that the plasmid-encoded LA adhesin involved in the first stage of adhesion is fimbrial in nature (Knutton *et al.*, 1987a). This observation contrasts with those from previous studies (Scotland *et al.*, 1983a; Levine *et al.*, 1985; Andrade and Santa Rosa, 1986b). It was suggested that a small number only of organisms in an EPEC culture are fimbriate which may explain why the fimbriae are difficult to detect. Both fimbriae and outer-membrane proteins may be involved in the first stage of adherence and it is

likely that chromosomal genes are involved in the second adhesion stage. The nature of this adhesion, however, and the factors responsible for microvillous disruption are not yet known.

Adherence and pathogenesis

Although EPEC strains possess toxic and adhesive capabilities which are likely to be involved in the disease process, it has been proposed that the intimate attachment of EPEC strains to intestinal mucosa could disturb the function of the microvillous border and bring about diarrhoea (Moon *et al.*, 1983). Evidence that adhesive non-toxigenic organisms can cause disease has been demonstrated in a distinct class of strains of *E. coli* that show HEp2 adherence but which give negative results in tests for VT, LT and ST. These strains of *E. coli* do not belong to the EPEC serotypes and are designated enteroadherent *E. coli* (EAEC) (Mathewson *et al.*, 1985). In epidemiological studies they have been shown to be associated with diarrhoea and their pathogenicity has been confirmed in human volunteers (Mathewson *et al.*, 1986). Diarrhoea caused by EAEC strains is generally less severe than that caused by EPEC strains. As some EPEC strains do not produce detectable Shiga toxin (Marques *et al.*, 1986), they may in fact resemble EAEC strains. It should be noted, furthermore, that HEp2 adhesion in EAEC is distinct from that of EPEC strains (Levine, 1987).

The role of the LA adhesin in disease has been clearly demonstrated. Nevertheless, because strains of EPEC lacking the EAF plasmid can still cause mild diarrhoea in volunteers, it may be that the plasmidless organisms retain some adhesive capacity. The demonstration by Knutton *et al.* (1987a) that these plasmidless strains are, nevertheless, capable of attaching to epithelia and effacing microvilli is in line with this idea.

Strains of *E. coli* belonging to non-EPEC serogroups but possessing the LA adhesin occur but, on the basis of epidemiological studies, are thought not to be pathogenic; they presumably lack other virulence attributes (Levine, 1987), suggesting that adhesion alone is insufficient to bring about diarrhoea.

Other virulence factors

Although toxin production and adhesive capabilities are probably the major virulence determinants of EPEC strains, other factors are likely to be involved in helping the organism reach and multiply

at the site of infection, and in evasion of host defences.

O antigen. It has been demonstrated that both ETEC and EPEC strains have neutral O-antigens but do not have K-polysaccharide capsules, whereas strains belonging to other pathogenic groups of *E. coli* have either acidic O-antigens or charged capsular antigens (Jann and Jann, 1985). With EPEC strains restricted to certain O types, it is possible that the O-antigen has an important, but as yet unidentified, role in EPEC virulence. Possible roles include the ability to take up plasmid-mediated virulence factors or evasion of host defences. In support of this is the finding of Sakazaki *et al.* (1974) that a rough EPEC strain was avirulent in the RIL whereas the smooth parent strain was virulent.

In a strain of *E. coli* that was pathogenic for rabbits and that produces lesions similar to those given by EPEC *in vivo*, it has been shown that the O-antigen allows bacteria to survive in the intestine by preventing their uptake by the M cells of Peyer's patches (Inman *et al.*, 1986).

Although the O antigen may be important, it has been shown that within an EPEC O-serogroup only certain O:H combinations are enteropathogenic (Wachsmuth, 1980); thus, the O-antigen alone does not specify virulence and other factors must be involved.

Mucinase. Ross (1959) demonstrated mucinase activity in EPEC strains of various serotypes but not in normal-flora strains of *E. coli*. Similar activity has been demonstrated in *V. cholerae* (Finkelstein *et al.*, 1983). The mucinase enzyme of this organism has been shown to be active against lactoferrin, mucin and fibronectin and this ability may contribute to pathogenesis. Further work must be carried out with EPEC strains to detect and characterise mucin-degrading enzymes that might be involved in the breakdown of mucous barriers, thereby allowing bacteria to approach the intestinal membrane.

Aerobactin production. Williams and Roberts (1985) first noted that EPEC strains could produce the iron-chelator aerobactin and suggested that it may be a virulence factor in EPEC strains as it is in other organisms, particularly in those involved in extra intestinal infection. Robins-Browne *et al.* (1985) could not detect aerobactin production in EPEC strains of proven virulence and argued against its role as a major virulence determinant.

In a later study, aerobactin production was detected in 19 of 51 EPEC strains, whereas none of 19 ETEC strains produced aerobactin (Roberts *et al.*, 1986); similar unpublished findings have been

obtained in this laboratory. There is obviously a major difference in the iron metabolism in these two groups of *E. coli*, related possibly to the avidity of adhesion and the environment in which the organism must grow. EPEC strains adhering intimately to the intestinal mucosa may encounter the iron-binding protein lactoferrin (Roberts *et al.*, 1986).

Thus, although aerobactin production is not essential for EPEC virulence, it may confer some advantages *in vivo*. Since the production of the Shiga-like toxin is influenced by iron, the ability to take up and utilise this element may be important to EPEC virulence. This is currently under investigation.

Invasive capabilities. Epithelial-cell invasiveness as detected by the ability of the organism to cause keratoconjunctivitis in the guinea-pig eye (Sereny test) is absent in EPEC strains (Goldschmidt and DuPont, 1976; Levine *et al.*, 1978). It has been demonstrated that EPEC adhesion to HEp2 cells is strongly dependent upon bacterial viability and prior cultivation in Ca-containing media. Similar findings are seen with strains of *Salmonella* and *Shigella* which, after attachment to animal cells, are internalised (Andrade and Santa Rosa, 1986a). There is evidence that such a process occurs after EPEC adherence to HEp2 cells and intracellular multiplication has been seen to take place (Andrade and Santa Rosa, 1986b). Although invasion is known to occur in tissue culture and in animal tissue (Moon *et al.*, 1983, Knutton *et al.*, 1987b), EPEC invasion of human-intestinal tissue *in vivo* has not been demonstrated (Rothbaum *et al.*, 1982, 1983).

Conclusion

Virulence in EPEC strains is clearly multifactorial and there is a great deal yet to be learnt about the relative importance of the various factors discussed. The production of a Shiga-like toxin (with both cytotoxic and enterotoxic properties) is likely to be of major importance and so too is intestinal adhesion.

Adhesion is poorly understood at present and two stages have been identified; there are at least two distinct kinds of adhesion in the first stage (LA and DA). The factors involved in the second stage, and those leading to microvillous disruption, are unknown. It may prove inappropriate to regard EPEC as a homogeneous group of organisms. If different virulence factors are produced by strains of different serotypes, this will necessitate subdivision based on virulence mechanisms.

Study of virulence factors has important practical implications. For vaccine production, we need to know which determinants are important in the causation of disease so that an effective vaccine may be developed. Diagnosis of disease may be simplified and improved if specific virulence factors can be detected in faecal samples or in strains of *E. coli* isolated from faeces.

Until tests such as DNA probes become commercially available, O-serotyping of strains of *E.*

coli remains the only method for routine detection of EPEC strains in the clinical microbiology laboratory. Positive reactions must be confirmed by tube agglutination with specific antisera and, where possible, by H-agglutination tests.

I should like to thank Drs H. R. Ingham and K. M. Wilkie and my colleagues in the Microbiology Department of Freeman Hospital for advice and constructive criticism during the preparation of this paper.

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