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Colonization factors of diarrheagenic *E. coli* and their intestinal receptors

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While *Escherichia coli* is common as a commensal organism in the distal ileum and colon, the presence of colonization factors (CF) on pathogenic strains of *E. coli* facilitates attachment of the organism to intestinal receptor molecules in a species- and tissue-specific fashion. After the initial adherence, colonization occurs, and the involvement of additional virulence determinants leads to illness. Enterotoxigenic *E. coli* (ETEC) is the most extensively studied of the five categories of *E. coli* that cause diarrheal disease, and has the greatest impact on health worldwide. ETEC can be isolated from domestic animals and humans. The biochemistry, genetics, epidemiology, antigenic characteristics, and cell and receptor binding properties of ETEC have been extensively described. Another major category, enteropathogenic *E. coli* (EPEC), has virulence mechanisms, primarily effacement and cytoskeletal rearrangement of intestinal brush borders, that are distinct from ETEC. An EPEC CF receptor has been purified and characterized as a sialidated transmembrane glycoprotein complex directly attached to actin, thereby associating CF-binding with host-cell response. Three additional categories of *E. coli* diarrheal disease, their colonization factors and their host cell receptors, are discussed. It appears that biofilms exist in the intestine in a manner similar to oral bacterial biofilms, and that *E. coli* is part of these biofilms as both commensals and pathogens.

Keywords: colonization factors; intestinal receptors; diarrheagenic *E. coli*; enterotoxigenic *E. coli*; fimbriae; adhesins

Introduction

Escherichia coli is a Gram-negative, facultatively anaerobic rod, commonly found in the lower bowel of mammals and birds. The presence of *E. coli* in water and food is an indicator of fecal contamination. Dr Theodor Escherich, a German pediatrician, first identified *Bacillus coli commune* (now *Escherichia coli*) from normal infant feces in 1885 [43]. Escherich initially considered *E. coli* a commensal, but later identified it as a cause of urinary tract infections. Further investigations have implicated *E. coli* in sepsis and newborn meningitis (for review of colonization factors of extraintestinal *E. coli* see [61]). *E. coli* was first implicated as a causative agent in diarrheal disease in 1945 [15] after *Shigella* and *Salmonella* were ruled out in the indentification of pathogenic isolates from infants. *E. coli* diarrheal disease (ECDD) occurs world-wide, with deaths approaching 1 million per year. ECDD is primarily a problem in infants and children in developing countries, but adult travelers to those countries are also at risk. In developed countries, small outbreaks of ECDD occur in child care settings, nursing homes and restaurants in which improperly prepared food is served.

Five categories of diarrheagenic *E. coli* are currently recognized [95,167]: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAggEC), and enteroinvasive *E. coli* (EIEC). This categorization is based on virulence properties of the bacteria, such as elaboration of toxins and CF, and/or specific types of interactions with intestinal epithelial cells.

Two central themes of microbial pathogenicity are that (i) pathogenic bacteria must attach to a eucaryotic cell surface, and (ii) this attachment involves interaction of an adhesin molecule with a receptor molecule [49]. By convention, the adhesin molecule is present on the microbe and the receptor molecule on the host cell surface. The terms colonization factor (CF) and colonization factor antigen (CFA) applied to ETEC, denote adhesins that promote the colonization of host tissues. The term putative colonization factors (PCF) is applied to adhesins for which a specific role in colonization has yet to be determined. CS (coli surface antigen) is an additional term in the *E. coli* CF literature, and has been used to describe individual subcomponents of CFA/II and CFA/IV, or more recently identified CF, such as CS7 and CS17. CF is a functional term that encompasses all members of this group of adhesin and putative adhesin molecules.

Adhesins take several forms, most often as distinct morphological structures called fimbriae (also known as pili) and fibrillae, but also as afimbrial, surface protein molecules that have not been directly visualized. Fimbriae are non-flagellar filamentous appendages composed of repeat-

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Abbreviations: CF, colonization factor; CFA, Colonization Factor Antigen; CS, coli-surface-associated antigen; EAggEC, enteroaggregative *E. coli*; ECDD, *E. coli* diarrheal disease; EHEC, enterohemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; Gal, galactose; GalNAc, *N*-acetyl galactosamine; LT, heat-labile toxin; NeuAc, *N*-acetyl neuraminic acid; PCF, Putative colonization factor; RBC, red blood cells; SLT, Shiga-like toxin; ST, heat-stable toxin

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ing protein subunits. They tend to be rigid rods of 4–10 nm diameter that are either evenly distributed on the surface of the bacterial cell (peritrichous) or found at one end of the cell (polar). The molecular weight of the subunits of ECDD CF fall within the range 14 000–27 000 Da (Table 1). In most cases, ECDD fimbriae are peritrichous, extending outward from the cell surface, with diameters of 6–8 nm, but bundle-forming pili (BFP) of EPEC [39,53,141], and Longus, an ETEC fimbria [55] are polar fimbriae. In contrast to fimbriae, fibrillae are 2–4 nm in diameter, and are either long and wiry or curly and flexible.

Adhesive properties of *E. coli* were first recognized in the early 1900s with the observation of hemagglutination, the adherence to and clumping of red blood cells (RBC) by certain strains of *E. coli*, but fimbriae were not associated with this hemagglutination activity until 1955 [41]. Hemagglutination has proven useful for the detection of CF and for the identification of pathogens, but may also be useful in the determination of the CF carbohydrate receptor specificity. Assessment of binding to additional cell types and to tissues has provided an additional link to their role in virulence. The identification of specific receptor molecules in cell and tissue binding has lagged behind the body of data specifically on CF. The CF and the receptor molecules are both logical targets for inhibiting the interaction between pathogen and host cell. Thus, the use of CF, CF analogs, receptors, or receptor analogs could prevent attachment of pathogens [134]. Alternatively, antibody to CF could block the initial adherence to the host cell. In fact, vaccine efforts focused on anti-CF immunity have been successful in farm animals [108] and are currently being evaluated in clinical trials in humans [146].

The study of bacteria present along the length of the alimentary tract [stomach, small intestine (duodenum, jejunum, ileum), and large intestine (proximal to distal colon)] shows that quantitative and qualitative differences exist. Although reports of the actual numbers of bacteria present at each region of the intestine vary, there is consensus that the numbers and variety of bacteria in the stomach, duodenum, and jejunum are low, are intermediate in the ileum, and in the colon are very high [83,109]. Variations in collection and culture methods used may account for the reported qualitative and quantitative differences; in particular, variations may be due to improvements in the isolation and culture of anaerobes [109]. Several mechanisms appear responsible for maintaining lower numbers of bac-

Table 1 Morphologic and size characteristics of colonization factors

CF (species)[CFA]	Morphology	Diameter	Subunit MW[a]	Ref
EPEC				
BFP	fimbrial, rod	ND[b]	18734 D	[39,53,141]
AF/R1 (rabbit)	fibrillar, rod	2–3 nm	16527 D	[7][c]
EHEC				
unnamed	fimbrial, rod	ND	16000 P	[79]
EAggEC				
AAF/I	fibrillar, rod	2.5 nm	15601 D	[110, 115]
ETEC: Non-human				
K88ab; ac; ad (pig)	fibrillar, rod	2.1 nm	27539 D; 27328 D, 27533 D	[78]
K99 (calf, lamb, pig)	fibrillar, rod	3.0 nm	16534 D	[123]
987P (pig)	fibrillar, rod	2–3 nm	17204 D	[33]
ETEC: Human				
CFA/I [I]	fimbrial, rod	7 nm	15074 D	[45,82]
CS1 [II]	fimbrial, rod	7 nm	15246 D	[118]
CS2 [II]	fimbrial, rod	7 nm	15421 M	[25]
CS3, CS3a[d] [II]	fibrillar, flexible	2–3 nm	15112 D & 15246 M	[25,70,96]
CFA/III [III]	fimbrial, rod	7–8 nm	25309 D	[66,149]
CS4 [IV]	fimbrial, rod	6–7 nm	14961 M	[25,164]
CS5 [IV]	fimbrial, flexible	5–6 nm	18617 D	[29,99]
CS6[e] [IV]	undetermined[f]	—	15058 D & 15877 D	[e]
CS7	fibrillar, helical	3.5–6.5 nm	21500 P	[100]
CS17	fimbrial, rod	6–7 nm	17500 P	[100]
PCFO9 [CS13]	fibrillar, flexible	unknown	27000 P	[100]
PCFO20	fimbrial, rod	7 nm	25000 P	[156]
PCFO148	fibrillar, curly	3 nm	unknown	[87]
PCFO159	fimbrial, rod	6–7 nm	18600 P	[145]
PCFO166	fimbrial, rod	6–7 nm	15500 P & 17000 P	[100]
2230	undetermined[f]	—	16000 P	[30]
8786	undetermined[f]	—	15349 D	[2,3]
Longus	fimbrial, rod	7 nm	22000 P	[55]

[a]Molecular weight determination: D, calculated from deduced amino acid sequence derived from DNA sequence; M, determined by electrospray mass spectrometry ([25] and Cassels and Pannell, unpublished); P, estimated by SDS-PAGE
[b]ND, not determined
[c]Also Cassels, unpublished, and Cantey, unpublished
[d]CS3: two distinct polypeptides have been detected by protein sequencing [63] and mass spectrometry ([25] and Cassels and Pannell, unpublished)
[e]CS6: two distinct polypeptides have been detected by DNA and protein sequencing (Wolf and Gaastra, unpublished)
[f]Undetermined: surface-detected, though non-fimbrial and non-fibrillar

teria in the small intestine: acidic pH in the stomach, peristalsis, and the gut associated mucosal immune system, especially secretory immunoglobulin A. When any of these mechanisms is impaired, malabsorption and diarrhea may occur [128]. Qualitatively, the genera and species of bacteria change throughout the alimentary tract. In general, lactobaccilli, streptococci, and staphylococci predominate in the stomach and duodenum, whereas streptococci, *Bacteroides*, and lactobaccilli predominate in the jejunum and proximal ileum. In the distal ileum and colon, the predominant isolates are *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Peptostreptococcus*, and *E. coli*. Significant numbers of *E. coli* generally are not found until the distal ileum and colon; even there, *E. coli* are vastly outnumbered by other bacteria. The CF of ETEC and EPEC allow attachment and colonization of the bacteria in the small intestine, where they cause toxic effects or intestinal cell damage. EHEC, EAggEC, and EIEC adhere to the colon, where either toxic effects or invasion with cell damage occur.

The intestinal flora, including *E. coli*, appear to constitute a biofilm, particularly through the use of CF for adherence and colonization, with similarities to biofilms of dental plaque bacteria (see Cassels *et al* [24], and others, this volume) and biofilms of *Pseudomonas aeruginosa* in the respiratory tract [120]. In this review, we briefly describe the major categories and pathogenic and virulence properties of organisms responsible for ECDD, and we describe the CF associated with each category of ECDD, their genetics, and the receptors for the CF.

ETEC

ETEC is an important cause of traveler's diarrhea and causes an estimated 800000 infant deaths per year worldwide [10]. ETEC diarrhea is common in areas where fecal contamination of water and food occurs. Symptoms of ETEC infection result from heat labile toxin (LT) and/or heat stable toxin (ST) that cause net fluid secretion in the intestine. LT is closely related to cholera toxin, both structurally and functionally [60], and the symptoms of traveler's diarrhea and cholera are similar, although diarrhea caused by ETEC is generally less severe. A dose of 10^8 to 10^{10} ETEC is necessary to cause diarrhea, as determined in human volunteer studies [146]. Typically, untreated infections of travelers resolve in about one week when the mucosal immune system clears the pathogen. Studies in volunteers have demonstrated that protection against reinfection with a homologous strain occurs after initial ETEC infection [94]. The importance and prevalence of ETEC were recognized in the late 1960s, and a large body of information on the epidemiology, genetics, biochemistry, and immunochemistry of ETEC CF has accumulated. Thus, ETEC will be covered in greatest depth in this review.

Eighteen CF from human ETEC have been described (Table 1), and this number is likely to increase. Although they are named as colonization factors, their role in *E. coli* colonization in humans has not been rigorously tested. Some CF have been tested in animal models; others have been regarded as CF based on their binding to cells or tissues (Table 2).

The first CF to be described from a human ETEC was CFA/I. It was from a strain recovered from a patient with severe cholera-like diarrhea in Dacca, Bangladesh [47]. CFA/I is a rigid fimbrial rod, with a diameter of 7 nm (Table 1) which functions as a CF in infant [47] and adult rabbits [1]. The second CF from ETEC was named CFA/II [46], but it was later found to be a mixture of either CS1 and CS3, or CS2 and CS3 [140]. CS1 and CS2 are rigid rods with diameters of 7 nm, but CS3 is a flexible fibrilla with a diameter of 2–3 nm. CFA/III was described in 1984 and is a fimbria with a diameter of 7–8 nm [66]. CFA/IV [152] was found to be a mixture of CS4 and CS6, CS5 and CS6, or CS6 alone [153]. CS4 and CS5 are fimbriae, but the structure of CS6 is undetermined [88,164]. CS6 is a CF in the reversible intestinal tie adult rabbit diarrhea model [144]. These are the most-studied CF from human ETEC.

Additional fimbrial (CS17, PCFO20, CFO159, and PCFO166), fibrillar (CS7, PCFO9, and PCFO148) and undetermined (22230 and 8767) CF have been identified (Tables 1 and 2). The names of these CF from ETEC have not continued the pattern of 'CFA' (Table 1). Often they are first named 'PCF' as putative colonization factors which may then be superseded by another name. For instance, CFA/IV was first called PCF8775 [106], and PCFO9 is also called CS13 [32]. The lack of systematic naming is unfortunate and not easily remedied.

Most of the human ETEC CF have demonstrated binding activities (Table 2) by the hemagglutination of bovine, human, guinea pig and chicken RBC, and/or by the binding of bacteria to human enterocytes or to human cell lines. Mannose is routinely added to inhibit hemagglutination by Type 1 fimbriae (not to be confused with CFA/I), the common or somatic fimbriae which are produced by almost all *E. coli* [85], but have no clear role in pathogenesis.

The study of the domestic animal ETEC strains bearing K88, K99 and 987P (Tables 1 and 2) has provided a valuable contribution to the ETEC literature. These CF and additional domestic animal ETEC CF, such as F41, CS31A, CS1541, F17, F42, F141, F165, 2134P, and 8813 have been reviewed elsewhere [32,108].

ETEC: Phenotype and distribution

Characteristics of ETEC from Asia [6,26,57,104, 105,133,154], South America [9,59,104], the Mid East [166], Africa [104] and Europe [11,12,56] have been published, but surveys of ETEC have not been geographically comprehensive, resulting in underrepresentation of certain areas. These ETEC were screened for CF, O:H serotype and toxins. Screening for CF was limited to those that had been identified at the time of the study, and CF to which antibody or DNA probes were utilized by the investigator. Most studies included the identification of CFA/I, CS1, CS2, CS3, CS4, CS5, and CS6.

CFA/I, CS3, and CS6 have been found as the sole CF on many ETEC, but with rare exceptions, CS1 is found only with CS3, CS2 with CS3, CS4 with CS6, and CS5 with CS6. In one survey from the Middle East these seven CF were detected on 75% [166] of the ETEC while in a survey from Southeast Asia, these CF accounted for only 23% [154]. Indeed, the lack of known CF on ETEC in

Table 2 Binding characteristics of colonization factors of ECDD organisms

CF (species)[CFA]	Hemagglutination[a]	Tissue/cell line[b]	Ref
EPEC			
BFP	Hum O	HEp-2	[53]
AF/R1(rabbit)	None	Rab BB	[28]
EHEC			
unnamed	None	Henle 407	[79]
EAggEC			
AAF/I	Hum A, Rat, Mo	HEp-2, Colonocytes	[89,110,115]
ETEC: Non-human			
K88ab (pig)	Chk, Pig, Rab	Pig BB, Hum Ent	[8, 158]
K88ac (pig)	GPig	Pig BB, Hum Ent	[8,158]
K88ad (pig)	Pig, GPig	Pig BB, Hum Ent	[8,158]
K99 (calf, lamb, pig)	Hum, Shp, Hor, GPig	Pig BB	[158]
987P (pig)	None	Rab BB, Pig BB	[36]
ETEC: Human			
CFA/I [I]	Bov, Hum A, Chk	Hum Ent, Caco-2	[27,31,44]
CS1 [II]	Bov[c]	Hum Ent, Caco-2[d]	[31,86]
CS2 [II]	Bov	Hum Ent	[86,138]
CS3, CS3a [II]	Bov	Hum Ent, HT-29	[86,112]
CFA/III [III]	None	Hum Ent	[66,88]
CS4 [IV]	Bov, Hum A	Hum Ent	[88,152]
CS5 [IV]	Bov, Hum A	Hum Ent	[88,99]
CS6 [IV]	None	HeLa	[59]
CS7	Bov, Hum, GPig	Hum Ent	[100]
CS17	Bov	None	[100]
PCFO9 (CS13)	Hum, Chk	Hum Ent, Buc Eph	[100]
PCFO20	n.d.[e]	Caco-2	[156]
PCFO148	None	Hum Ent	[87]
PCFO159	None	n.d.[e]	[145]
PCFO166	Bov, Hum	Hum Ent	[100]
2230	None	Hum Ent, Caco-2	[30,31]
8786	Bov, Hum	Hum Ent, Caco-2	[2]

[a]Mannose-resistant hemagglutination: Bov, Bovine; Chk, chicken; GPig, guinea pig; Hor, Horse; Hum A, human A; Hum O, human O; Hum, human (type not given), Mo, mouse; Shp, sheep
[b]Rab BB, rabbit intestinal brush borders; Pig BB, pig intestinal brush borders; Hum Ent, human enterocyte binding; Buc Eph, buccal epithelial cells
[c]Ryu and Cassels, unpublished
[d]Hum Ent, adherent to human enterocytes by a strain also bearing CS3
[e]n.d., not done

these studies has led to discovery of new CF such as PCFO166 [101], CS17 [103], and PCFO159 [145]. Perhaps testing for these CF will increase the number of ETEC with defined CF.

For many years, serotyping was the only means of identifying pathogenic *E. coli* and data on O:H serotypes demonstrate the variety of ETEC. Some combinations of antigens are common on ETEC (Table 3), but 110 combinations have been reported from the geographic areas cited at the beginning of this section. A given CF may be expressed in a variety of serotypes; 37 O:H serotypes have been reported with CFA/I, 35 O:H serotypes with CS6, and 19 O:H serotypes with CS3. Why certain specific combinations of antigens are widespread, and thus not randomly distributed, and also how the antigens may interact for enhanced virulence is not known. Clearly though, ETEC have a large variety

Table 3 Characteristics of most commonly reported ETEC[a]

CF	Serotype	Toxin	Distribution
CFA/I	O78:H12	LTST or ST	Asia, South America
CFA/I	O126:H12	ST	Asia
CFA/I	O128:H12	LTST or ST	Asia, Mid East
CFA/I	O153:H45	ST	Mid East, Europe, South America
CS3/CS1 or CS3/CS2	O6:H16	LTST	Asia, Mid East, South America
CS3	O8:H9	LTST	Asia, Mid East, South America
CS6 or CS6/CS4	O27:H7	ST	Mid East, Africa, South America
CS6	O148:H28	ST	Asia, Mid East, South America
CS6	O169:H[b]	ST	Asia, South America
CS17	O8:H9	LT	Asia, Africa, South America

[a]Reported from at least three countries
[b]H serogroups undefined or nonmotile

of surface antigens with O serogroups showing the greatest variation, followed by H serogroups, CF, and toxins.

Only a few CF of EPEC, EHEC, and EAggEC have been described and their distribution has not been studied as thoroughly as ETEC, so it remains to be seen if the CF of these pathogenic *E. coli* are as varied and widespread as those from ETEC.

EPEC

EPEC primarily affect infants under one year of age, with infection resulting in diarrhea with mucus, vomiting, fever and malaise that may persist for weeks [40]. A global distribution of EPEC is reported, including reports from child-care settings in the United States [40]; a high infant mortality due to EPEC in developing countries is common. The pathogenesis of EPEC has been conceptualized as a three-stage process: (1) localized adherence, the fimbrial-mediated stage of attachment to intestinal epithelial cell brush borders; (2) effacement of brush border microvilli, including the cytoskeletal rearrangement of the brush border, with sloughing and elongation of the microvilli, hence the characteristic attaching and effacing lesions; and (3) the close attachment stage, with the accumulation of actin and other cytoskeletal proteins under the bacterial cell resulting in the appearance of a cup-like pedestal seen in electron micrographs [40]. So far, two virulence factors have been identified which may have roles in EPEC attachment to epithelial cells. Initial adherence appears to be the result of bundle-forming pilus (BFP)-mediated attachment [40,53], and an integral membrane protein of 94 kD, termed intimin, is involved in the close attachment stage of EPEC pathogenesis [72]. In studies of adult volunteers, EPEC instilled directly into the duodenum, but not into the large intestine, caused diarrhea [90,91], suggesting that specific receptor molecules for initial fimbrial-mediated attachment may be present in the duodenum but not in the colon.

Two CF from EPEC have been described. The first is the bundle-forming pilus (BFP) [39,54,157], named for its characteristic association into bundles as seen by electron microscopy. BPF are responsible for the first stage of EPEC pathogenesis mentioned in the preceding paragraph and for localized adherence in tissue culture assays. BFP occur in human EPEC of a variety of serotypes that cause localized adherence, but do not occur on EHEC, ETEC, or EIEC [52,141]. AF/R1, the second EPEC CF is a CF from strain RDEC-1, causing a diarrheal illness in rabbits that resembles EPEC infection in humans [17,147,148]. AF/R1 is an adhesin that imparts species-specificity for rabbit intestine [7,68,98,121,135] and has been shown to be a virulence factor for RDEC-1 *in vivo* [18,163]. If AF/R1 is replaced by CF specific for human tissue, RDEC-1 interacts with cultured cells derived from humans just as EPEC do [19,71]. AF/R1 have not hemagglutinated any tested RBC, but do adhere to rabbit small intestine brush borders and isolated microvilli, while EPEC expressing BFP hemagglutinate human O RBC and bind to HEp-2 cells (Table 2).

EHEC

In most cases, EHEC cause a non-bloody diarrhea lasting for about one week, but EHEC disease may progress to hemorrhagic colitis and some individuals develop hemolytic-uremic syndrome (HUS), a condition characterized by renal failure, anemia, and severe thrombocytopenia. EHEC disease causes an estimated 200–400 deaths annually in North America and has been reported from multiple continents. Young children and elderly adults seem to be especially susceptible to EHEC. Ingestion of contaminated food, primarily ground beef, is the most common route of infection. Serotype O157:H7 accounts for 80% of the reported cases, but testing for other serotypes has been spotty and they may be more common than is presently appreciated. Less than 100 bacterial cells of O157:H7 are needed to induce disease in humans, reflecting a high infectivity and virulence and accounting for outbreaks. EHEC reside as commensals in the alimentary tract of cattle, sheep and goats. Initially reported in 1982, EHEC incidence reporting has risen sharply in recent years. EHEC possess at least two unique putative virulence determinants: (1) fimbriae that mediate attachment to intestinal epithelial cells, (2) cytotoxins similar to Shiga toxin in structure and function (Shiga-like toxin; SLT). EHEC, like EPEC, induce the three stages of attachment, effacement and close adherence to host intestinal epithelium and express the intimin protein. The potent SLT produced by EHEC is believed to be responsible for the severe clinical manifestations, hemorrhagic colitis and HUS. For a current review of this important pathogen, see [114].

A fimbria from EHEC serotype O157:H7 has been purified and partially characterized (Tables 1 and 2) [79]. This fimbria is encoded on a 60-MDa plasmid, is a rigid rod with subunit molecular mass of 16 000 Da, and does not cross react antigenically with CFA/I or BFP. This EHEC fimbria did not hemagglutinate a panel of RBC, including human A, bovine, guinea pig, horse, cow, and chicken, but mediated adherence to Henle 407 intestinal epithelial cells.

EAggEC

A group of *E. coli* isolated from children with persistent diarrhea (greater than 14 days duration) fall into a category of ECDD organisms termed EAggEC. EAggEC adhere to cultured HEp-2 cells in a 'stacked-brick' appearance and adhere to human colonic mucosa. Two putative virulence determinants are characteristic of EAggEC: a bundle-forming fimbria responsible for the aggregative adherence, termed AAF/I, and a novel heat-stable enterotoxin. EAggEC primarily affects children from less-developed countries, although EAggEC has been isolated in Great Britain [131].

Knutton *et al* [89] studied 44 EAggEC strains for hemagglutination, HEp-2 cell binding, and binding to human intestinal cells. Forty-three strains hemagglutinated RBC, and 43 strains bound to human colonocytes but not to duodenal enterocytes. By electron microscopy, four different fimbrial types were identified on this group of EAggEC, including AAF/I [89]. AAF/I, the only EAggEC fimbria isolated and characterized to date, has been cloned and sequenced [129], and is discussed below.

EIEC

EIEC is not a common cause of diarrhea, except in Brazil (for unknown reasons), although a few large outbreaks have occurred in more developed nations. Clinically, EIEC is indistinguishable from shigellosis with dysentery or profuse diarrhea, fever, abdominal cramps, and chills being prominent symptoms. EIEC invade and multiply inside enterocytes, kill the cells in a manner similar to *Shigella*, and share many biochemical, antigenic and genetic characteristics of *Shigella*. EIEC and *Shigella* both depend on a 140-MD plasmid coding for the production of several outer membrane proteins involved in invasiveness. No CF or receptors of EIEC have been identified. Refer to [62] for current review of EIEC.

Genetics of CF

Of the CF discussed in this review, the DNA sequences of all genes necessary for synthesis and assembly of seven CF, CFA/I [75], CS1 [51,77,118,132], CS3 [13,70], CS6 (Wolf and Gaastra, unpublished), K88 [4,5,67,107], K99 [4,122,124,125,137], and AAF/I [129] are known (Figure 1). In all cases, the genes for structural subunits and genes for assembly are transcribed in the same direction. The genes probably are in operons, with some mechanism for enhanced expression of the major structural subunit [76], although this has not been proved experimentally in all cases. DNA sequences limited to the genes for the structural proteins of CFA/III [149], CS5 [29], 8786 [3], BFP of EPEC [39,141], 987P [33], and AF/R1 ([165], Cantey, unpublished) have been determined. Most likely they are component parts of operons containing additional genes for assembly.

Analysis of the DNA sequence data suggests that there are four groups of ECDD CF. One group includes CS3, CS6, K88, K99, and AAF/I. They all have genes for structural subunits, chaperones for delivering the subunits across the periplasm in the correct conformation, and ushers to deliver subunits to the bacterial surface, where they may be inserted into the growing structure (Figure 1). This scheme has been well defined for K88 and K99 [4] as well as for P fimbriae that occur on *E. coli* from urinary tract infections and are the prototype for this group [73]. There are conserved patterns of amino acids at the C-terminus of structural subunits utilized in interactions with the chaparone protein, but the subunit proteins are antigenically distinct [136].

CFA/I and CS1 are members of a different group. The DNA sequences of genes for subunits and accessory genes have significant homology distinct from the above group and DNA hybridization data suggest CS4 and PCFO166 are also members of this group [143]. Limited antigenic cross reactivity [102,126,127] of the proteins may be a result of similarities in the N-terminal sequences (Figure 2a). The DNA and protein data taken together suggest the group includes CFA/I, CS1, CS2, CS4, CS17, and PCFO166.

CFA/III [149] and BFP [39,141] belong to neither of these groups, but their DNA sequences show they are members of the Type IV pili family [149]. Type IV pili are distinguished by a unique signal sequence on the structural subunit gene that functions in export to the outer surface of the bacterial cell and by a characteristic amino acid sequence at the N-terminus. Type IV fimbriae have been found in *Vibrio cholerae*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, and a few other Gram-negative bacteria [151]. Another Type IV pilus has been found in ETEC and named Longus [55]. The DNA sequence of Longus is not available, but the amino acid sequence of the first 20 residues is identical to CFA/III. It is possible that Longus is CFA/III, but the geographic distribution of Longus compared to CFA/III suggests they are distinct CF [55,149]. See Figure 2b for comparison of the N-terminal sequences of these three *E. coli* Type IV fimbriae from *E. coli* that cause diarrheal disease.

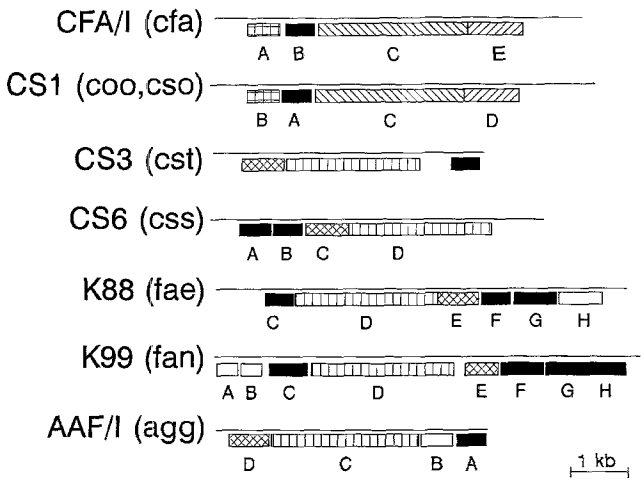

Figure 1 Maps of genes for CF expression. Gene names are given in parentheses. Transcription is from right to left, filled boxes indicate structural subunits and genes with significant homologies are represented with like patterns: ⊞ accessory protein, ▨ assembly protein, ▨ assembly protein, ▩ chaperone, ▥ usher, □ undefined function, kb, kilobase pairs

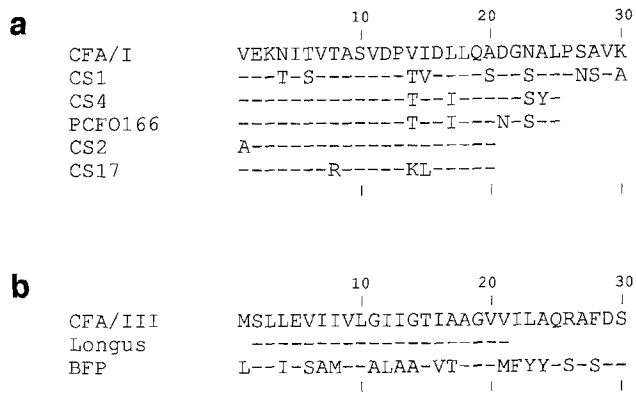

Figure 2 (a) N-terminal sequences of CF in ETEC group consisting of CFA/I, CS1, CS4, PCFO166, CS2, CS17. Hyphens indicate amino acid identity with CFA/I. Sequence references: CFA/I [23,82], CS1 [118], CS4 ([164] and Cassels and Carter, unpublished), PCFO166 [143], CS2 [84], and CS17 [92]. (b) N-terminal sequences of Type IV fimbriae of diarrheagenic *E. coli*. Hyphens indicate amino acid identity with CFA/III. Sequence references: CFA/III [149], Longus [55], and BFP [39]

CS5 may be unique and not belong to any of these families. Only the structural subunit has been characterized [29], but the DNA sequence of the structural subunit is not similar to the group that includes CFA/I and also lacks the conserved pattern characteristic of Type IV pili or the C-terminus for interaction with chaperones, although there is some homology with F41 [29]. There is antigenic cross reactivity between CS5 and CS7 [64], so these may be members of the same group.

Regulation of CF expression is currently being defined, both in terms of environmental stimuli and mechanism of regulation. It has been known for some time that CF are expressed at 37° C and less well at lower temperatures [34,140]. The usual growth medium for expression of ETEC is CFA agar plates, but some require bile salts [100,143]. CFA/I expression is repressed by glucose [81] and high iron concentrations [80]. Expression of K99 is reduced in the presence of leucine via Lrp [14], a regulator of a number of diverse proteins in *E. coli* [113]. Expression of AAF/I (measured by a marker gene) is enhanced at low pH, low iron concentrations, anaerobiosis, and growth temperatures of 30° to 37° C [111]. There are no systematic studies that have determined whether these conditions regulate expression of other CF.

Proteins that function as positive regulators increase expression of CF: CFA/I is regulated by CfaD [130], (also called CfaR [22]), CS1 by Rns [20], CS4 by CsvR [35] and CsfR (Wolf, unpublished), and AAF/I by AggR [111]. The DNA sequences of the five genes for these regulatory proteins are very closely related, and DNA hybridization experiments have shown that homologous genes are present in ETEC expressing CFA/I, CS1, CS2, CS4, and PCFO166 [21,65,142], suggesting that all CF in this group share a common regulator. The common regulator hypothesis has been confirmed by demonstrating that a positive regulator transferred from an ETEC expressing one CF can enhance expression of another CF [22,35,58,111, 130,143,162]. To date there is no published evidence that the positive regulators affect expression of fimbriae other than those in the CFA/I group in ETEC, although AggR from EAggEC enhances expression of AAF/I [111].

The mechanism for positive regulation by these proteins is currently under investigation. The proteins bind to DNA in a way that enhances transcription of their target genes. They belong to a family of positive regulators that include araC, rhaR, rhaS, toxT, appY, and virF, which are positive regulators for expression of a variety of genes in *E. coli*, *Vibrio cholerae*, *Shigella*, and *Yersinia enterocolitica* [20,130].

Temperature regulation of CFA/I expression is mediated through the interaction of CfaD, a protein named H-NS, and the promoter DNA that is upstream of the CFA/I genes [74]. Rns has been found to be a positive regulator of its own expression by interfering with negative regulation by an unknown factor [50]. How these positive regulators respond to environmental signals known to regulate CF is not understood.

Receptors for CF

Receptor molecules for *E. coli* were among the first specific CF receptor molecules identified. P fimbriae of uropathogenic *E. coli* utilize glycolipids containing the Galα(1–4) Gal sequence, while S fimbriae of *E. coli* meningitis strains utilize glycoproteins with terminal NeuAcα(2–3)Gal as receptor molecules. Most of the work on identification and characterization of CF receptors comes from non-target tissues, such as erythrocyte inhibition or recognition of a glycoprotein or glycolipid from a non-target tissue (Table 4). Methods are available to detect receptor-mediated binding of the bacteria to a glycoprotein after electrotransfer to nitrocellulose from a polyacrylamide gel, and to detect binding to glycolipids separated on thin-layer chromatograms with an overlay of bacteria (Table 4). Although receptor molecules can be isolated from erythrocytes and other cells, and the fine specificity of the adhesin interaction with the receptor determined, the receptor molecule in the true target tissue likely will not be identical. With carbohydrate microheterogeneity commonly occurring in glycoproteins, the carbohydrate portion in a 'true' receptor molecule will be similar but may not be identical to that isolated from non-target tissue. The protein portion of the molecule may in particular bear little resemblance to the receptor protein of the target tissue. Microheterogeneity is not a concern in the situation where a glycolipid is identified as a receptor molecule, but a glycolipid receptor found in a non-target tissue may not be present in the target tissue. While the information obtained from the identification of receptors of non-target tissue is of value, especially for CF carbohydrate specificity determination, purification and characterization of receptors from target tissues yield the data of greatest biological and pathogenic significance.

Several studies have been done on receptors isolated from the true target tissue. For example, both glycoprotein and glycolipid receptors from pig intestine that are specific for K88ac, K99 and 987P have been purified and partially characterized. In addition, the receptor for AF/R1 has been purified and characterized from rabbit small intestine. Characteristics of the AF/R1 receptor suggest that it contributes to EPEC pathogenesis in an interesting way as is discussed below. No specific receptor molecules have been identified for CF from EHEC, EAggEC, or EIEC.

Human ETEC receptors

Both glycoprotein and glycolipid receptor molecules for CFA/I have been identified from human cells (Table 4). The 26-kD glycoprotein glycophorin A was purified from RBC and identified as the receptor molecule [119]. GM2 inhibition of hemagglutination was used by Faris *et al* [48] to identify a glycolipid receptor for CFA/I, and they showed that sialidase treatment of RBC abolished binding of CFA/I positive bacteria. GM2 adsorbed to gold particles was shown in electron micrographs to bind directly to CFA/I [16].

In a preliminary study, Orø *et al* [116], identified asialo-GM1 as a glycolipid receptor for CS1, CS2, CS3 and CS4. Adherence to glycoproteins from rabbit intestine and human intestinal cell line HT-29 has also been examined; a rabbit protein of 120–140 kD recognized by CS3, CS7

Table 4 Detection and identification of colonization factor receptor molecules

Colonization factor	Detection of adherence[a]	Receptor type	Receptor identity[b]	Carbohydrate specificity[b]	Ref
EPEC					
AF/R1	Rab intestine	glycoprotein	130 and 140 kD	NeuAc	[121]
ETEC: Non-human					
K88ac	Pig intestine	glycoprotein	210 & 240 kD	unknown	[42]
K88ab	TLC assay	glycolipid	Galβ1-Cer, others	Gal β1-	[117]
K99	Horse RBC	glycolipid	hematoside	NeuGc	[139]
	Pig intestine	glycolipid	NeuGc-GM_3	unknown	[150]
987P	Rab intestine	glycoprotein	14 kD	GlcN, GalN, ManN	[37]
	Piglet intestine	glycolipid	lac-cer, sulfatide	unknown	[38]
ETEC: Human					
CFA/I	Human RBC	glycoprotein	glycophorin A	NeuAc	[119]
	HA inhibition	glycolipid	GM_2	unknown	[48]
	Blot binding	protein	HT 29 30–35 kD	NeuAc	[160]
CS1	TLC assay	glycolipid	asialo-GM_1	unknown	[116]
CS2	HA inhibition	unknown	unknown	NeuAc	[138]
	TLC assay	glycolipid	asialo-GM_1	unknown	[116]
CS3	TLC assay	glycolipid	asialo-GM_1	unknown	[116]
	Blot binding	protein	HT 29 30–35 kD	NeuAc	[160]
	Blot binding	protein	120–140 kD	GalNAcβ1-4Gal	[161]
CS4	TLC assay	glycolipid	asialo-GM_1	unknown	[116]
CS7	Blot binding	protein	Rab BB 120–140 kD	unknown	[159]
CS17	Blot binding	protein	Rab BB 120–140 kD	unknown	[159]

[a]TLC assay, overlay of bacteria onto glycolipids separated by thin-layer chromatography; HA inhibition, hemagglutination inhibition; Blot binding, overlay of bacteria onto proteins separated by SDS-PAGE and electrotransfer to nitrocellulose
[b]Glycolipids: Cer, ceramide; hematoside, Neu5Gcα2-3Gal-β1-4Glc-β1-1 ceramide; lac-ser, lactosylceramide- Galβ1-4Glcβ1-1 ceramide; sulfatide, SO_3Galβ1-1 ceramide
[c]Carbohydrate abbreviations: NeuAc, *N*-Acetyl neuraminic acid; Gal, galactose; HA, hemagglutinin; RBC, red blood cell; NeuGc, *N*-Glycolyl neuraminic acid; GlcN, *N*-Acetyl glucosamine; GalN, *N*-Acetyl galactosamine; ManN, mannosamine

and CS17 [159], and HT-29 proteins in the size range of 30–35 kD were recognized by CFA/I and CS3 [160]. The binding of CFA/I and CS3 to the HT-29 proteins, but not CS3 binding to rabbit proteins, was abolished on treatment of the transblot with sialidase [160]. Further studies on the binding specificity of CS3 showed that binding of CS3 could be inhibited by the galactose-specific lectin from the plant *Maackia amurensis*, and by GM1, asialo-GM1, and GM2 gangliosides [161]. The common carbohydrate moiety of the glycolipids, ie GalNAcβ1-4Gal, was found to bind to CS3-expressing bacteria, and when immobilized on nitrocellulose, CS3-expressing bacteria adhered to it [161].

Human EPEC receptors

BFP, the EPEC fimbria responsible for localized adherence [39], hemagglutinate human O RBC and bind to cultured HEp-2 cells (Table 2), but a specific receptor molecule has not been identified. Strain E2348/69 (O127:H6) and other EPEC strains responsible for localized adherence bind to glycolipids containing the GalNAcβ1-4Gal binding epitope [69]. Additional localized adherent strains of EPEC not tested in the above study adhered to Chinese hamster ovary cells in a lactosamine Galβ(1–4 or 1-3)GlcNAcβ dependent manner and caused actin accumulation and invasion of the cells [155]. While the data appear to be inconsistent, additional studies should resolve whether BFP is responsible for either binding activity, and whether additional adhesins are involved in EPEC initial stage adherence.

AF/R1 receptor

The receptor for the AF/R1 pilus-mediated adherence of RDEC-1 has recently been purified from enterocytes of rabbit small intestine and characterized as a three-component glycoprotein complex linked to the cytoskeleton [93,121]. Rafiee *et al* [121], identified the receptor by demonstrating that radiolabeled RDEC-1 adhered to ileal brush borders and microvilli, but not to the terminal web. The receptor could then be released and purified from microvilli only by treatments that indicated the receptor was intimately associated with the cytoskeleton (detergent with high salt or detergent and ATP). The role of carbohydrate, and in particular sialic acid, in RDEC-1 binding to brush borders was demonstrated by the diminished binding to brush borders on pretreatment with periodate and by a dramatic reduction in binding by pretreatment with sialidase or inhibition of the interaction with free sialic acid [121]. The addition of free AF/R1 fimbriae or antibody to the fimbriae blocked binding, demonstrating that AF/R1 was responsible for RDEC-1 binding. The complex consists of two glycoproteins of 130 and 140 kD and a third protein of 130 kD, which appears to be brush border myosin 1. Apparently, the two glycoproteins span the membrane, with the extracellular portion exposing the carbohydrate needed for AF/R1 binding, and the intracellular portions are firmly attached to the cytoskeleton via brush border myosin 1. It is unknown at present what normal cellular role this receptor complex plays, but adherence of AF/R1 to this receptor provides close access of the RDEC-1 bacteria to the cyto-

skeleton and probably contributes to the dramatic rearrangement seen in stage 2 and stage 3 EPEC disease. AF/R1 are not necessary for the cytoskeletal rearrangement since a mutant lacking AF/R1 still makes A/E lesions *in vivo*, but the mutation clearly results in attenuation [161].

Conclusions

Several CF from *E. coli* associated with diarrheal disease have been identified. Characterization of these CF is rather uneven, both in terms of structure and function as adhesions. Some CF, particularly those recently identified, have been only partially characterized. Others, such as CFA/I, K88, and K99, have been studied in detail, so their structure, genetics, carbohydrate binding properties, and receptors are known. With the genetic data has come an appreciation for the biogenesis of CF, including the proteins involved and their specific roles, as well as the primary structure of each of the proteins. From the primary structure of CF, immunochemical and functional interpretations are possible. In contrast, the literature covering receptors of CF is not nearly as complete, primarily because of the difficulty in obtaining appropriate tissues as source material. Only AF/R1, a fimbria from an EPEC that is restricted to rabbits, has been associated with a receptor that has an association with the pathogenic process. We anticipate that as more receptors are identified and extensively characterized, and the glycoprotein and glycolipid topology of the intestine more extensively mapped, a greater appreciation for the molecular basis of adhesion of bacteria to intestine and better overall understanding of the microbial ecological environment of the gut will result. Of significant additional value may be future discoveries leading to treatment and prevention of disease caused by *E. coli*.

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