MINIREVIEW

Diffusely adherent *Escherichia coli* strains expressing Afa/Dr adhesins (Afa/Dr DAEC): hitherto unrecognized pathogens

Chantal Le Bouguénec[1,2] & Alain L. Servin[1,2]

[1]Unité de Pathogénie Bactérienne des Muqueuses, Institut Pasteur, Paris, France; and [2]Unité 756 Physiopathologie des Cellules Epithéliales, Institut National de la Santé et de la Recherche Médicale, Faculté de Pharmacie, Université Paris XI, Châtenay-Malabry, France

Correspondence: Chantal Le Bouguénec, Unité de Pathogénie Bactérienne des Muqueuses, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France. Tel.: +33 1 40613280; fax: +33 1 40613640; e-mail: clb@pasteur.fr

Received 6 January 2006; revised 11 January 2006; accepted 11 January 2006.
First published online 6 February 2006.

doi:10.1111/j.1574-6968.2006.00144.x

Editor: Ian Henderson

Keywords
pathogenic *Escherichia coli*; diffuse adherence; Afa/Dr adhesins.

Abstract

Diffusely adherent *Escherichia coli* (DAEC) strains are currently considered to constitute a putative sixth group of diarrheagenic *E. coli*. However, on the basis of their diffuse adherence to HEp-2 and HeLa cells, the detection of *afa/dra/daa*-related operons encoding this adherence phenotype, and the mobilization of decay-accelerating factor, both commensal and pathogenic strains can be classified as Afa/Dr DAEC isolates. Furthermore, strains associated with diarrheal diseases and strains causing extra-intestinal infections can also be identified as Afa/Dr DAEC strains. Although several cell signaling events that occur after epithelial cells have been infected by Afa/Dr DAEC have been reported, the pathophysiological processes that allow intestinal and extra-intestinal infections to develop are not fully understood. This review focuses on the genetic organization of the *afa/dra/daa*-related operons and on the virulence factors that trigger cellular responses, some of which are deleterious for the host cells. Finally, this review suggests future lines of research that could help to elucidate these questions.

Introduction

Escherichia coli is a commensal bacterium of the human gastrointestinal tract. It is found in the mucus layer lining the epithelium of the intestine, and is generally symbiotic with its host. However, the high plasticity of the genome of this bacterial species gives it a tremendous capacity to evolve, leading to the emergence of pathogenic strains from the commensal strains. The wide diversity of virulence factors has led to the definition of numerous pathogenic groups (or pathovars), which exhibit differing physiopathological behavior patterns. Pathogenic *E. coli* strains are the main cause of bacterial diarrhea worldwide, and of extra-intestinal infections, of which urinary tract infections (UTIs), meningitis and septicemia are the most severe. The virulence factors of several enteric pathovars of *E. coli* responsible for episodes of diarrhea have been extensively investigated, providing some insight into the molecular mechanisms underlying diarrhea (Nataro & Kaper, 1998; Kaper *et al*., 2004). However, for a long time, little was known about the pathogenic mechanisms of other pathovars, and their role in causing diarrheic syndromes remained to be demonstrated. This was true, in particular, of *E. coli* strains displaying diffuse adhesion [diffusely adherent *Escherichia coli* (DAEC) strains], which are associated with the watery diarrhea that can become persistent in young children (Nataro & Kaper, 1998; Le Bouguenec, 1999; Kaper *et al*., 2004; Servin, 2005). DAEC strains are a heterogeneous group of isolates, all of which exhibit diffuse adherence (DA) to epithelial cells in the classical laboratory assay of adherence to HEp-2 or HeLa cells (Cravioto *et al*., 1991). In many cases, the DA pattern of DAEC isolates is due to the production of adhesins encoded by a family of *afa/dra/daa*-related operons (Table 1). The *afa* operon (Labigne-Roussel *et al*., 1984) was the first to be characterized and sequenced, and the second was the *dra* operon (Nowicki *et al*., 1987). Unlike the virulence factors from pathovars associated with severe acute diarrhea (enterohemorragic, enterotoxigenic, and enteroinvasive *E. coli*), and the virulence factors that specify only extra-intestinal *E. coli* isolates (Oelschlaeger *et al*., 2002; Johnson, 2003) that all require or prefer a particular genetic background to arise, the *afa/dra/daa* operons are genes that arise and are expressed in a variety of genetic backgrounds (Escobar-Paramo *et al*., 2004a).

Table 1. Adhesins encoded by *afa/dra/daa*-related gene clusters in human pathogenic *Escherichia coli*

Pathotype	Adhesin subtype	Operon	Identified cellular receptor(s)	Pathogenicity	Reference strain	Morphology
DAEC	AfaE-I	*afa-1*	DAF	D, UTI	KS52	A/F
	AfaE-II	*afa-2*	DAF	D, UTI	A22	A
	AfaE-III*	*afa-3*	DAF, CEACAM1, CEA, CEACAM6	D, UTI	A30	A
	AfaE-V	*afa-5*	DAF	D, UTI	AL851	A
	Dr*	*dra*	DAF, CEACAM1, CEA, CEACAM6, collagen type-4	UTI	IH11128	F
	Dr-II	*dra2*	DAF	UTI	7732	A
	F1845	*daa*	DAF, CEACAM1, CEA, CEACAM6	D, UTI	C1845	F
	Nfa-1	*nfa-1*	DAF, CEA†	UTI	827	A
ExPEC	AfaE-VIII	*afa-8*	Unknown	UTI	AL862	A

DAEC, diffusely adherent *E. coli*; ExPEC, extra-intestinal pathogenic *E. coli*; D, diarrhea, UTI, urinary tract infections; A, afimbrial; F, thin fimbrial; DAF, decay-accelerating factor; CEACAM, carcinoembryonic, antigen-related molecule.

**afaE3* PCR also detects the Dr adhesin-encoding gene (*draE*), which is 99.4% identical to *afaE3* (Le Bouguenec *et al*., 1993).

†J. Guignot, I. Kansau, A.L. Servin (pers. commun.).

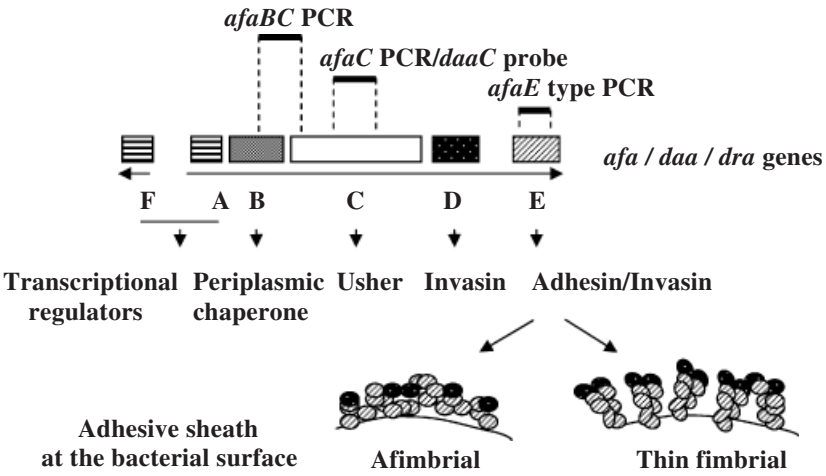

Fig. 1. Genetic and structural organization of the *afa*-related gene clusters (*afa*, *daa*, and *dra* operons). Boxes represent genes and letters designate the different genes. The arrows refer to the direction of transcription of the genes. The DNA fragments specifying the operons are indicated; the *afaBC* PCR specifies operons encoding Afa/Dr adhesins; the *afaC* PCR and *daaC* probe specifies all operons encoding Afa-related adhesins (including the *afa-8* operon); six *afaE*-type PCRs specify the *afaE1*, *afaE2*, *afaE3*, *afaE5*, *afaE8*, and *daaE* genes (Le Bouguenec *et al*., 2001). The afimbrial or fimbrial structure of the adhesive sheath depends on the *afaE* sequence.

The Afa/Dr family of adhesins

Some adhesins, known as the afimbrial or nonfimbrial adhesins, have long been associated with an amorphous, outer membrane-associated structure on the surface of *Escherichia coli* strains that cause diarrhea and UTIs (Soto & Hultgren, 1999). The first determinant to be identified that encodes an adhesin that is not associated with visible fimbriae on the bacterial surface was the *afa-1* (for afimbrial adhesin) operon, which was isolated in 1984 from a uropathogenic *E. coli* (Labigne-Roussel *et al*., 1984). Since then, various operons with very similar genetic organizations and closely related at the DNA level have been described in strains that cause both intestinal and extra-intestinal infections (Le Bouguenec *et al*., 1993; Garcia *et al*., 1994, 1996) (Fig. 1). Unlike the other *afa* genes, the *afaE* gene is highly heterogeneous, leading to the production of antigenically distinct adhesins. The *afa* family of gene clusters includes the *afa* operons described in both diarrheal and uropathogenic *E. coli* (Labigne-Roussel *et al*., 1984; Le Bouguenec *et al*., 1993; Zhang *et al*., 1997). For example, strains that only express this virulence factor have been reported to be causative agents of mild chronic diarrhea, especially in children over 2 years of age in developing countries, and of UTIs. Interestingly, an *afa*-positive clone without any other virulence factor has been reported to cause both diarrhea and cystitis in a child (Germani *et al*., 1997). Information deduced from the analysis of the sequence of the *afa-3* gene cluster indicates that the operons of the *afa* family are composed of six genes (*afaA* to *afaF*) and organized to form two distinct transcriptional units, consisting of *afaA* to *afaE*, and *afaF*, respectively (Le Bouguenec *et al*., 1993; Garcia

et al., 1996). Other related operons have also been reported, including the *dra* operons detected in uropathogenic isolates (Nowicki *et al*., 1987; Pham *et al*., 1997), the *daa* operon reported in a diarrheagenic strain (Bilge *et al*., 1989), and the *nfa* operon found in a uropathogenic isolate (Ahrens *et al*., 1993). Consequently, the probes (Bilge *et al*., 1989; Nowicki *et al*., 1989) and PCR (Le Bouguenec *et al*., 1992, 2001) assays based on the sequence of the *daaC* and *afaB-C* genes also detect the presence of all the operons in the family.

The gene subtypes *afaE1*, *afaE2*, *afaE3* (and *draE*, which is 99.4% identical with *afaE3*), *afaE5*, *dra*, *dra2*, *daa*, and *nfa* encode adhesins designated as Afa/Dr adhesins (Le Bouguenec *et al*., 2001; Nowicki *et al*., 2001; Servin, 2005). These adhesins are expressed by *E. coli* strains of human origin, and have been demonstrated to recognize the Cromer blood group antigen Dr(a) on the human decay-accelerating factor (DAF, CD55) as a receptor (Nowicki *et al*., 1990; Pham *et al*., 1997), a characteristic that enables the bacteria to promote agglutination of human erythrocytes even in the presence of mannose (a property known as 'mannose-resistant hemagglutination'). They have been found in isolates from both diarrheagenic and uropathogenic clones, indicating that, regardless of their adhesin-encoding gene subtype, strains that produce Afa/Dr adhesins may cause both intestinal and extraintestinal infections. The *afaE1*, *afaE3*, and *afaE5* subtypes are more prevalent than the *afaE2* and *daa* subtypes in diarrheagenic strains. The distribution of *afaE* subtypes in *afa*-positive isolates from patients with pyelonephritis may differ in different parts of the world (Ishitoya *et al*., 2003). AfaE adhesins expressed by *E. coli* from animal species that do not recognize the human DAF have also been reported; they are encoded by the *afa-7* and *afa-8* operons (Lalioui *et al*., 1999; Lalioui & Le Bouguenec, 2001). The *afaE7* subtype has been only identified in one pathogenic *E. coli* isolate from an animal species. In contrast, the *afaE8* subtype that was first identified in clinical isolates from animals was subsequently associated with human pathogenic *E. coli* strains. It has never been detected in any isolates from human diarrhea, but was identified as the most predominant *afaE* subtype in uropathogenic *E. coli* (Le Bouguenec *et al*., 2001).

The *afa/dra/daa* operons are also interesting, because they reportedly code for both afimbrial (such as AfaE-I and AfaE-III) and fimbrial (such as F1845 and Dr) adhesive structures on the bacterial surface (Labigne-Roussel *et al*., 1984; Nowicki *et al*., 1987; Bilge *et al*., 1989; Garcia *et al*., 1994; Pham *et al*., 1997). Various studies have attempted to find out how these adhesins assemble to form surface organelles with different morphologies. Microscopic examination of chimeras between the *afa-3* and *daa* operons encoding afimbrial and fimbrial structures, respectively, indicated initially that the morphology of the adhesive architecture depends on the sequence of the adhesin-encoding gene (Garcia *et al*., 1996). Recent work combining biochemical and structural studies of AfaE-III has shown that the afimbrial pattern of AfaE adhesins results from a collapse of the fine fimbrial structures on the bacterial surface (Anderson *et al*., 2004a; Pettigrew *et al*., 2004). Interestingly, the AfaE-III thin filament provides the link between the bacterial usher (AfaC) and the invasin (AfaD) at the tip. Assembly of AfaE-III (Anderson *et al*., 2004a) and Dr adhesins (Piatek *et al*., 2005) involves mechanisms similar to those reported for numerous other adhesive fimbriae, designated as donor strand complementation and donor strand exchange mechanisms (Sauer *et al*., 1999).

Other virulence factors

Although a variety of virulence genes from different pathogenic *Escherichia coli* strains and serotypes have been observed in DAEC from both patients with intestinal and extra-intestinal infections and healthy controls, no secretion system-encoding genes have been found associated with pathogenic Afa/Dr DAEC isolates (Blanc-Potard *et al*., 2002). However, cellular lesions observed in Afa/Dr DAEC-infected intestinal cells were clearly adhesin independent, suggesting the presence of additional virulence factors (Peiffer *et al*., 2000a, 2001). The *sat* gene has been found in an Afa/Dr strain isolated from a child with diarrhea, and which expresses the AfaE-V adhesin (Taddei *et al*., 2003, 2005).

It has been clearly demonstrated that the flagella is an important virulence factor involved in proinflammatory responses, such as the induction of IL-8 production, that follow infection by enteropathogenic *E. coli* (EPEC) (Zhou *et al*., 2003), enteroaggregative *E. coli* (EAEC) (Khan *et al*., 2004; Harrington *et al*., 2005), and enterohemorrhagic *E. coli* (Berin *et al*., 2002; Khan *et al*., 2004), and diffusely adhering *E. coli* (Betis *et al*., 2003b). Interestingly, it has recently been reported that motile Dr and F1845-positive *E. coli* strains were more able to induce IL-8 production than nonmotile strains (Arikawa *et al*., 2005), suggesting that the flagellum plays an important role in Afa/Dr DAEC-induced proinflammatory responses (Tieng *et al*., 2002; Betis *et al*., 2003a, b).

The interaction between Afa/Dr adhesins and membrane-bound receptors leads to cell signaling

The Afa/Dr adhesins from human pathogenic *Escherichia coli* recognize human DAF (Afa/Dr_{DAF}) (Nowicki *et al*., 1990, 1993, 2001) as a cellular receptor. Interestingly, Dr adhesin does not recognize rat, mice, guinea pig or pig DAF (Hudault *et al*., 2004). DAF, a 70 kDa glycoprotein, is one of the cell membrane proteins that regulate the complement

cascade, and is widely distributed in hematopoietic cells, intestinal and urinary epithelia, and endothelial cells (Lublin *et al.*, 2000). The SCR3 domain of DAF, which is important in regulating the complement cascade, is involved in Afa/Dr adhesin binding (Nowicki *et al.*, 1993). Two different, although closed, regions of the SCR3 of DAF, approximately 20 Å apart, are involved in both these events (Hasan *et al.*, 2002; Pettigrew *et al.*, 2004). Afa/Dr adhesin binding results in a dense accumulation of DAF molecules beneath adherent bacteria that could be detected by a fluorescent DAF-staining test (Goluszko *et al.*, 1999; Le Bouguenec *et al.*, 2001; Goluszko *et al.*, 2001a) (Fig. 2). DAF is known to have signal transduction capacity.

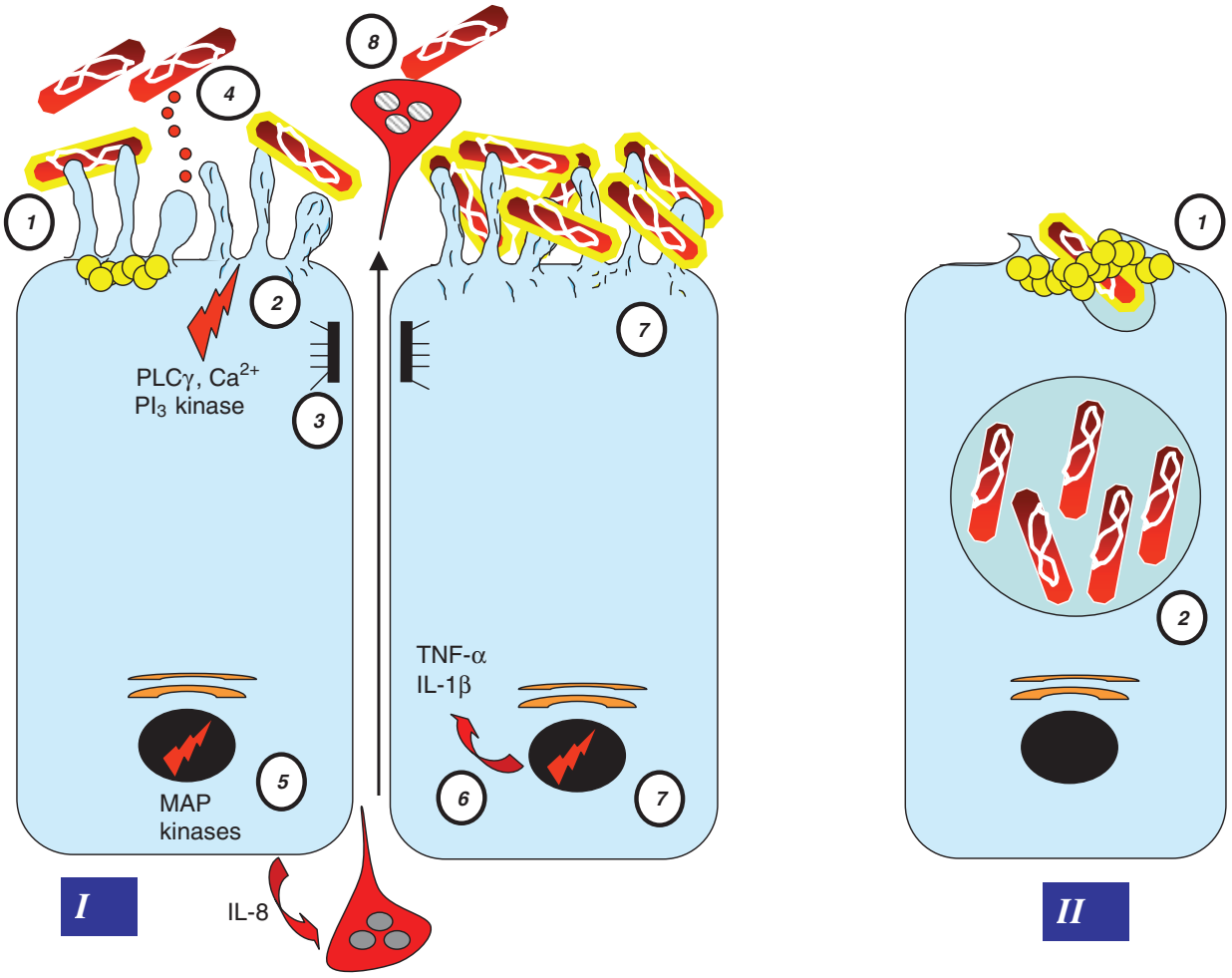

Fig. 2. Interaction of Afa/Dr diffusely adherent *Escherichia coli* (DAEC) strains with epithelial cells. In *I*, interaction with fully differentiated, polarized epithelial cells. Bacteria expressing Afa/Dr adhesins interacted with membrane-bound receptors, including the recognition of decay-accelerating factor (DAF) by Afa/Dr_{DAF} adhesins (AfaE-I, AfaE-II, AfaE-III, AfaE-V, Dr, Dr-II, Nfa-1, and F1845) and carcinoembryonic, antigen-related molecule-1 (CEACAM1), CEA, and CEACAM6 by Afa/Dr_{CEA} adhesins (AfaE-III, Dr, and F1845) (1). The structural and functional lesions induced included a loss of microvilli resulting from a signaling pathway involving protein tyrosine kinase(s), phospholipase Cγ, phosphatidylinositol 3-kinase, protein kinase C, and an increase in $[Ca^{2+}]_i$ that controls the rearrangements of brush border-associated F-actin and villin cytoskeletal proteins, and a decrease in the expression and enzyme activities of functional brush border-associated proteins, including sucrase-isomaltase, dipeptidylpeptidase IV, the glucose transporter SGLT1, and the fructose transporter GLUT5 (2). Changes in the distribution of tight junction-associated proteins lead to an increase in paracellular permeability (3). Afa/Dr DAEC isolates produce the secreted autotransporter toxin, Sat, inducing marked fluid accumulation in the intestine (4). The induced transepithelial migration of polymorphonuclear leukocytes (PMNLs) results from the production of the proinflammatory cytokine IL-8 as a result of activating a MAP kinase-dependent signaling pathway (5). In turn, PMNL transmigration promotes the production of the proinflammatory cytokines TNF-α and IL-1β (6) that induce the upregulation of DAF and MHC class I chain-like gene A (7). Transmigrated PMNLs showed an increased apoptosis (8). In *II*, interaction with undifferentiated cells. The internalization of Afa/Dr DAEC occurs by a mechanism involving lipid rafts and dynamic microtubules (1). Recognition of DAF and/or $\alpha_5\beta_1$ integrin by Afa/Dr adhesins and/or invasins plays a pivotal role in bacterial internalization (1). Internalized Afa/Dr DAEC strains survived within a large vacuole (2).

Some carcinoembryonic, antigen-related molecules (CEACAMs) including CEACAM1, CEA, and CEACAM6, also act as receptors for a subfamily of Afa/Dr adhesins (designated as Afa/Dr_{CEA}) (Guignot *et al.*, 2000; Berger *et al.*, 2004). This binding, like that to DAF (Goluszko *et al.*, 1999; Guignot *et al.*, 2000), induces the recruitment of CEACAM molecules around adhering bacteria (Guignot *et al.*, 2000; Berger *et al.*, 2004) in detergent-insoluble microdomains, which is consistent with a role of lipid rafts in the pathogenicity of Afa/Dr strains (Goluszko *et al.*, 1997a; Guignot *et al.*, 2001; Kansau *et al.*, 2004). Recognition of DAF, CEA, and CEACAM6, but not of CEACAM1, is accompanied by the induction of microvilli extensions at the cell surface that resemble those observed by Cookson & Nataro (1996), promoting tight attachment of bacteria. The induced microvilli extensions are controlled by signaling that includes the activation of Cdc42, a Rho GTPase, and the phosphorylation of ezrin/radixin/moesin (Berger *et al.*, 2004).

Dr adhesin is the only member of the Afa/Dr_{DAF} family to recognize type-IV collagen, and its binding is inhibited by chloramphenicol (Westerlund *et al.*, 1989; Carnoy & Moseley, 1997). Interestingly, the DraE fusion protein that binds to the DAF receptor does not bind to type-IV collagen (Van Loy *et al.*, 2002). Investigation of the precise atomic basis for the sensitivity of DraE binding to chloramphenicol demonstrates that, unlike other chloramphenicol-protein complexes, drug binding is mediated via recognition of the chlorine 'tail,' rather than via intercalation of the benzene rings into a hydrophobic pocket (Pettigrew *et al.*, 2004).

Internalization of Afa/Dr DAEC

It is important to mention that the *afa/dra/daa* operons constitute the first known example of a single operon promoting both adhesion to epithelial cells and invasion into these cells. Several reports have indicated that various Afa/Dr-positive strains are able to enter epithelial cells *in vitro* (Jouve *et al.*, 1997; Goluszko *et al.*, 1997a, 2001b; Selvarangan *et al.*, 2000; Guignot *et al.*, 2001; Plancon *et al.*, 2003). Afa/Dr-positive bacteria are not true invasive pathogens, as only a small percentage of bacteria adhering to the cells are internalized. Afa/Dr DAECs enter epithelial cells by a zipper-like mechanism (Jouve *et al.*, 1997; Kansau *et al.*, 2004) (Fig. 2). Internalization of Dr-positive bacteria is dependent on dynamic microtubules and lipid rafts (Goluszko *et al.*, 1997a; Guignot *et al.*, 2001; Kansau *et al.*, 2004). Despite the fact that Afa/Dr-positive bacteria fail to enter fully differentiated intestinal cells (Guignot *et al.*, 2001), *ex vivo* observation of the bacterial internalization phenomenon after infecting piglet ileal explants with *afa-3*-positive bacteria suggests that internalization may also occur *in vivo* (Humbert *et al.*, 2000). The characteristics of the entry of uropathogenic Afa/Dr-positive bacteria into epithelial cells are consistent with the possibility that intracellular bacteria could form an intracellular reservoir for subsequent cycles of infection (Plancon *et al.*, 2003). We do not yet fully understand the exact process(es) by which the Afa/Dr-positive strains are internalized. It is of interest that at least two Afa products (AfaD and AfaE) contribute to entry mechanisms. Working on the *afa-3* operon, we first proposed that AfaE-III is an adhesin and that AfaD-III is an invasin. AfaD entry is not dependent on an adhesion step mediated by the adhesin (Jouve *et al.*, 1997). The characteristics of AfaD, which was recently shown to be located only at the tip of the flexible fibrillar structure (Anderson *et al.*, 2004a) and to be able to detach from the bacterial surface (Jouve *et al.*, 1997), could possibly account for the low level of bacterial internalization, because the concentration of AfaD is important in the invasion process. AfaD-III (Garcia *et al.*, 1996, 2000) was the first demonstrated example of an invasin encoded by an *afa* operon. In addition to other AfaD subtypes (Garcia *et al.*, 1996, 2000; Zalewska *et al.*, 2001, 2005), this family of invasins includes proteins encoded by EAEC (Garcia *et al.*, 2000). The AfaD invasins, like the invasin from enteropathogenic *Yersinia* and the intimin produced by EPEC, bind to β1 integrins that are recruited at the bacterial–epithelial cell interaction sites (Guignot *et al.*, 2001; Plancon *et al.*, 2003; Kansau *et al.*, 2004). Interestingly, there have been several reports that the adhesin encoded by the *dra* operon is also involved in internalization of Dr-positive *Escherichia coli* (Goluszko *et al.*, 1997a, 2001b; Selvarangan *et al.*, 2000). Data obtained while investigating the role of the *dra* operon in bacterial internalization showed that the binding of Dr adhesin to DAF leads to the penetration of the Dr-positive bacteria into nonfusogenic intracellular vacuoles. Investigations of the DAF-binding site and the invasion-related domain in this Dr adhesin revealed that two distinct domains (the amino-terminal half and hydrophylic domain II, respectively) are implicated in these phenotypes (Anderson *et al.*, 2004b; Das *et al.*, 2005). It has been reported that a viable pool of intracellular Afa/Dr bacteria was maintained for several days after entry. Like type-1, fimbriae-producing *E. coli* strains internalized into bladder epithelial cells, intracellular Afa/Dr-positive bacteria are contained in inclusions within which they appear to be filamentous (Plancon *et al.*, 2003). These bacteria-containing vacuoles became so large after infection that it seemed unlikely that the epithelial cells affected could survive (Guignot *et al.*, 2001; Plancon *et al.*, 2003).

Pathogenesis of Afa/Dr DAEC strains associated with extra-intestinal infections

Urinary tract infections are among the most frequently acquired bacterial infections, and *Escherichia coli* accounts

for up to 90% of all UTIs (Zhang & Foxman, 2003). Several studies have strongly indicated that Afa/Dr-positive strains play an important role in the pathogenesis of UTIs. Such strains are especially common in pregnant women (Hart *et al.*, 1996, 2001), patients with recurring UTIs (Le Bouguenec *et al.*, 1992; Nowicki *et al.*, 1994; Usein *et al.*, 2001), and increase the risk of a second UTI (Foxman *et al.*, 1995; Zhang *et al.*, 1997). *Escherichia coli* strains bearing Dr adhesin display unique tropism to the basement membrane-renal interstitium that enables the bacteria to cause chronic pyelonephritis in experimental mice (Goluszko *et al.*, 1997b; Selvarangan *et al.*, 2004). Using an experimental model of mouse chronic pyelonephritis, it has been demonstrated that an isogenic mutant that did not produce the Dr adhesin was less virulent in causing chronic kidney infection in C3H/HeJ mice than the parental wild-type strain IH11128. Bacteria persistently colonized the renal interstitia of 50% of the animals tested for 1 year, with histological evidence of chronic pyelonephritis, whereas its isogenic Dr adhesin-negative mutant was gradually eliminated. It is not known whether the ability to produce Afa/Dr adhesins is associated with chronic bladder infection in mice. One interesting hypothesis is that the colonization of the mouse urinary tract occurs by a two-step process, initiated by binding to and penetration into urothelial cells, and is followed by translocation into the interstitial compartment and attachment to collagen fibers. Binding to type-IV collagen may enhance colonization, and thus further contribute to the spread of the infection (Selvarangan *et al.*, 2004). Using a pregnant rat model, the role of Afa/Dr adhesins in intrauterine infection during pregnancy was demonstrated, and it was clearly shown that both the adhesin (AfaE-III) and the invasin (AfaD-III) have an impact on the outcome of intratuterine infection (Wroblewska-Seniuk *et al.*, 2005).

Pathogenesis of Afa/Dr DAEC strains associated with intestinal disorders

The implication of Afa/Dr DAEC strains in diarrhea remains controversial. Interestingly, Afa/Dr DAEC strains have been implicated as a cause of diarrhea, especially in children, in both developing and developed countries (Giron *et al.*, 1991; Baqui *et al.*, 1992; Gunzburg *et al.*, 1993; Levine *et al.*, 1993; Germani *et al.*, 1996; Scaletsky *et al.*, 2002). It has already been shown that the relative risk of diarrhea associated with DAEC increases with the age of children, from 18 months to 5 years. The intestinal carriage of Afa/Dr DAEC strains has also been reported to be widespread in older children and adults. The consequences of this persistence in the intestinal mucosa are unknown. However, several observations have suggested a potential role in the development of chronic inflammatory intestinal diseases.

Recognition of DAF in infected epithelial cells as a result of Dr and F1845 adhesin binding is associated with cytoskeleton rearrangements implicating F-actin, villin, α-actinin, ezrin, and occasionally tropomyosin (Bernet-Camard *et al.*, 1996; Peiffer *et al.*, 1998; Goluszko *et al.*, 1999; Peiffer *et al.*, 2000b). In cultured intestinal cells forming a monolayer mimicking an epithelial intestinal barrier, Afa/Dr DAEC infection is followed by brush border lesions characterized by a loss of microvilli (Bernet-Camard *et al.*, 1996) (Fig. 2). The brush border lesion is the consequence of the disassembly of two major cytoskeleton proteins, F-actin and villin, as a result of the activation of Ca^{2+}-dependant signaling (Peiffer *et al.*, 2000b). In turn, the loss of brush border results from defective expression of brush border-associated functional intestinal proteins, including sucrase-isomaltase (SI), dipeptidylpeptidase IV (DPPIV), glucose transporter SGLT1, and fructose transporter GLUT5 (Peiffer *et al.*, 2000b). The lesion in sucrase-isomaltase is reinforced by the Afa/Dr DAEC-induced blockade of sucrase-isomaltase biosynthesis (Peiffer *et al.*, 2001).

Afa/Dr DAEC infection also leads to an increase in paracellular permeability and dramatic rearrangement of the distribution of the tight junction-associated proteins, ZO-1 and occludin, without affecting the transepithelial electrical resistance of the cell monolayer (Peiffer *et al.*, 2000a).

It was recently reported that the binding of the Afa/Dr adhesins to DAF in human intestinal cells is followed by a proinflammatory response (Tieng *et al.*, 2002; Betis *et al.*, 2003a, b; Arikawa *et al.*, 2005). Infection with an AfaE-III-positive *Escherichia coli* increases the expression of the major histocompatibility complex class-I-related MHC class I chain-like gene A (MICA), a molecule central in innate immune responses, and *in vitro* also triggers proinflammatory stimuli in intestinal cells (Tieng *et al.*, 2002). Moreover, infection with Afa/Dr-positive *E. coli* is followed by an increase in the production of the proinflammatory cytokine IL-8 (Betis *et al.*, 2003b; Arikawa *et al.*, 2005). Dr-induced IL-8 production results from the activation of mitogen-activated protein kinases, ERK1/2 mitogen-activated protein, P38, and Jun-C kinases (Betis *et al.*, 2003b). The basolateral secretion of IL-8 induces migration of polymorphonuclear leukocytes (PMNLs) across the epithelial barrier that promotes the production of TNF-α and IL-1β, which in turn promote the upregulation of DAF (Betis *et al.*, 2003a). Interestingly, Dr adhesin is released by the bacteria in response to multiple environmental signals, including anaerobiosis, indicating that bacteria could signal at distance for proinflammatory responses (Bouvet & Servin, pers. comm.). These findings are of particular interest, as both DAF and MICA are overexpressed on the surfaces of colonic epithelial cells from patients with Crohn's disease (Berstad & Brandtzaeg, 1998; Orchard *et al.*, 2001; Tieng *et al.*, 2002),

and positive-*daa*C strains have been isolated from the ileal mucosa of patients with Crohn's disease (Darfeuille-Michaud *et al*., 1998). Moreover, it has been observed that PMNL apoptosis is dependent on induction of their agglutination by Afa/Dr DAEC (Brest *et al*., 2004). The delivery of deleterious components, including proinflammatory cytokines, reactive oxygen species, and proteolytic enzymes by apoptotic PMNLs, could be a factor that increases the proinflammatory responses of Afa/Dr DAEC.

The evolution of Afa/Dr DAEC isolates and concluding remarks

Afa/Dr DAEC strains constitute a heterogeneous group of clones, since some that have been isolated from patients with either intestinal or extra-intestinal infections are recognized as true disease-causing agents, whereas others have been isolated from the normal stools of healthy people. The genetic basis of the virulence of clones obtained from clinical specimens has not yet been elucidated. An initial genomic approach, using the representational difference between a pathogenic, diarrhea-associated Afa/Dr DAEC strain, C1845, and a nonpathogenic K-12 isolate made it possible to identify short sequences (73 to 495) that are more prevalent among Afa/Dr clinical isolates than among non-Afa/Dr clinical isolates (Blanc-Potard *et al*., 2002). However, no gene-encoding factors that are potentially implicated in causing intestinal damage have been identified. The major virulence factor identified in Afa/Dr DAEC is encoded by the *afa/dra/daa* operons that have recently been divided into strains belonging to the four major phylogenetic groups of *Escherichia coli* (A, B1, B2, and D) (Escobar-Paramo *et al*., 2004a, b). A comparative genomic analysis of both commensal and pathogenic Afa/Dr DAEC strains belonging to each of these phylogenetic groups may be, in the coming years, the most informative approach for identifying Afa/Dr DAEC-specific pathogenic determinants and for obtaining a better understanding of the various pathophysiological processes implicated in the onset of both intestinal and extra-intestinal infections.

References

Ahrens R, Ott M, Ritter A, *et al.* (1993) Genetic analysis of the gene cluster encoding nonfimbrial adhesin I from an *Escherichia coli* uropathogen. *Infect Immun* **61**: 2505–2512.

Anderson KL, Cota E, Simpson P, Chen HA, Du Merle L, Bouguenec CL & Matthews S (2004a) Complete resonance assignments of a 'donor-strand complemented' AfaE: the afimbrial adhesin from diffusely adherent *E. coli*. *J Biomol NMR* **29**: 409–410.

Anderson KL, Billington J, Pettigrew D, *et al.* (2004b) An atomic resolution model for assembly, architecture, and function of the Dr adhesins. *Mol Cell* **15**: 647–657.

Arikawa K, Meraz IM, Nishikawa Y, Ogasawara J & Hase A (2005) Interleukin-8 secretion by epithelial cells infected with diffusely adherent *Escherichia coli* possessing Afa adhesin-coding genes. *Microbiol Immunol* **49**: 493–503.

Baqui AH, Sack RB, Black RE, *et al.* (1992) Enteropathogens associated with acute and persistent diarrhea in Bangladeshi children less than 5 years of age. *J Infect Dis* **166**: 792–796.

Berger CN, Billker O, Meyer TF, Servin AL & Kansau I (2004) Differential recognition of members of the carcinoembryonic antigen family by Afa/Dr adhesins of diffusely adhering *Escherichia coli* (Afa/Dr DAEC). *Mol Microbiol* **52**: 963–983.

Berin MC, Darfeuille-Michaud A, Egan LJ, Miyamoto Y & Kagnoff MF (2002) Role of EHEC O157:H7 virulence factors in the activation of intestinal epithelial cell NF-kappaB and MAP kinase pathways and the upregulated expression of interleukin 8. *Cell Microbiol* **4**: 635–648.

Bernet-Camard MF, Coconnier MH, Hudault S & Servin AL (1996) Pathogenicity of the diffusely adhering strain *Escherichia coli* C1845:F1845 adhesin-decay accelerating factor interaction, brush border microvillus injury, and actin disassembly in cultured human intestinal epithelial cells. *Infect Immun* **64**: 1918–1928.

Berstad AE & Brandtzaeg P (1998) Expression of cell membrane complement regulatory glycoproteins along the normal and diseased human gastrointestinal tract. *Gut* **42**: 522–529.

Betis F, Brest P, Hofman V, *et al.* (2003a) Afa/Dr diffusely adhering *Escherichia coli* infection in T84 cell monolayers induces increased neutrophil transepithelial migration, which in turn promotes cytokine-dependent upregulation of decay-accelerating factor (CD55), the receptor for Afa/Dr adhesins. *Infect Immun* **71**: 1774–1783.

Betis F, Brest P, Hofman V, *et al.* (2003b) The Afa/Dr adhesins of diffusely adhering *Escherichia coli* stimulate interleukin-8 secretion, activate mitogen-activated protein kinases, and promote polymorphonuclear transepithelial migration in T84 polarized epithelial cells. *Infect Immun* **71**: 1068–1074.

Bilge SS, Clausen CR, Lau W & Moseley SL (1989) Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. *J Bacteriol* **171**: 4281–4289.

Blanc-Potard AB, Tinsley C, Scaletsky I, *et al.* (2002) Representational difference analysis between Afa/Dr diffusely adhering *Escherichia coli* and nonpathogenic *E. coli* K-12. *Infect Immun* **70**: 5503–5511.

Brest P, Betis F, Cuburu N, *et al.* (2004) Increased rate of apoptosis and diminished phagocytic ability of human neutrophils infected with Afa/Dr diffusely adhering *Escherichia coli* strains. *Infect Immun* **72**: 5741–5749.

Carnoy C & Moseley SL (1997) Mutational analysis of receptor binding mediated by the Dr family of *Escherichia coli* adhesins. *Mol Microbiol* **23**: 365–379.

Cookson ST & Nataro JP (1996) Characterization of HEp-2 cell projection formation induced by diffusely adherent *Escherichia coli*. *Microb Pathogen* **21**: 421–434.

Cravioto A, Tello A, Navarro A, Ruiz J, Villafan H, Uribe F & Eslava C (1991) Association of *Escherichia coli* HEp-2 adherence patterns with type and duration of diarrhoea. *Lancet* **337**: 262–264.

Darfeuille-Michaud A, Neut C, Barnich N, *et al.* (1998) Presence of adherent *Escherichia coli* strains in ileal mucosa of patients with Crohn's disease. *Gastroenterology* **115**: 1405–1413.

Das M, Hart-Van Tassell A, Urvil PT, *et al.* (2005) Hydrophilic domain II of *Escherichia coli* Dr fimbriae facilitates cell invasion. *Infect Immun* **73**: 6119–6126.

Escobar-Paramo P, Clermont O, Blanc-Potard AB, Bui H, Le Bouguenec C & Denamur E (2004a) A specific genetic background is required for acquisition and expression of virulence factors in *Escherichia coli*. *Mol Biol Evol* **21**: 1085–1094.

Escobar-Paramo P, Grenet K, Le Menac'h A, *et al.* (2004b) Large-scale population structure of human commensal *Escherichia coli* isolates. *Appl Environ Microbiol* **70**: 5698–5700.

Foxman B, Zhang L, Tallman P, *et al.* (1995) Virulence characteristics of *Escherichia coli* causing first urinary tract infection predict risk of second infection. *J Infect Dis* **172**: 1536–1541.

Garcia MI, Labigne A & Le Bouguenec C (1994) Nucleotide sequence of the afimbrial-adhesin-encoding afa-3 gene cluster and its translocation via flanking IS1 insertion sequences. *J Bacteriol* **176**: 7601–7613.

Garcia MI, Gounon P, Courcoux P, Labigne A & Le Bouguenec C (1996) The afimbrial adhesive sheath encoded by the afa-3 gene cluster of pathogenic *Escherichia coli* is composed of two adhesins. *Mol Microbiol* **19**: 683–693.

Garcia MI, Jouve M, Nataro JP, Gounon P & Le Bouguenec C (2000) Characterization of the AfaD-like family of invasins encoded by pathogenic *Escherichia coli* associated with intestinal and extra- intestinal infections. *FEBS Lett* **479**: 111–117.

Germani Y, Begaud E, Duval P & Le Bouguenec C (1996) Prevalence of enteropathogenic, enteroaggregative, and diffusely adherent *Escherichia coli* among isolates from children with diarrhea in new Caledonia. *J Infect Dis* **174**: 1124–1126.

Germani Y, Begaud E, Duval P & Le Bouguenec C (1997) An *Escherichia coli* clone carrying the adhesin-encoding *afa* operon is involved in both diarrhoea and cystitis in twins. *Trans R Soc Trop Med Hyg* **91**: 573.

Giron JA, Jones T, Millan-Velasco F, *et al.* (1991) Diffuse-adhering *Escherichia coli* (DAEC) as a putative cause of diarrhea in Mayan children in Mexico. *J Infect Dis* **163**: 507–513.

Goluszko P, Popov V, Selvarangan R, Nowicki S, Pham T & Nowicki BJ (1997a) Dr fimbriae operon of uropathogenic *Escherichia coli* mediate microtubule-dependent invasion to the HeLa epithelial cell line. *J Infect Dis* **176**: 158–167.

Goluszko P, Moseley SL, Truong LD, *et al.* (1997b) Development of experimental model of chronic pyelonephritis with *Escherichia coli* O75: K5: H-bearing Dr fimbriae: mutation in the dra region prevented tubulointerstitial nephritis. *J Clin Invest* **99**: 1662–1672.

Goluszko P, Selvarangan R, Popov V, Pham T, Wen JW & Singhal J (1999) Decay-accelerating factor and cytoskeleton redistribution pattern in HeLa cells infected with recombinant *Escherichia coli* strains expressing Dr family of adhesins. *Infect Immun* **67**: 3989–3997.

Goluszko P, Selvarangan R, Nowicki BJ, Nowicki S, Hart A, Pawelczyk E & Nguyen K (2001a) Rapid receptor-clustering assay to detect uropathogenic and diarrheal *Escherichia coli* isolates bearing adhesins of the Dr family. *J Clin Microbiol* **39**: 2317–2320.

Goluszko P, Niesel D, Nowicki B, *et al.* (2001b) Dr operon-associated invasiveness of *Escherichia coli* from pregnant patients with pyelonephritis. *Infect Immun* **69**: 4678–4680.

Guignot J, Peiffer I, Bernet-Camard MF, Lublin DM, Carnoy C, Moseley SL & Servin AL (2000) Recruitment of CD55 and CD66e brush border-associated glycosylphosphatidylinositol-anchored proteins by members of the Afa/Dr diffusely-adhering family of *Escherichia coli* infecting the human polarized intestinal Caco-2/TC7 cells. *Infect Immun* **68**: 3554–3563.

Guignot J, Bernet-Camard MF, Pous C, Plancon L, Le Bouguenec C & Servin AL (2001) Polarized entry of uropathogenic Afa/Dr diffusely adhering *Escherichia coli* strain IH11128 into human epithelial cells: evidence for $\alpha 5\beta 1$ integrin recognition and subsequent internalization through a pathway involving caveolae and dynamic unstable microtubules. *Infect Immun* **69**: 1856–1868.

Gunzburg ST, Chang BJ, Elliott SJ, Burke V & Gracey M (1993) Diffuse and enteroaggregative patterns of adherence of enteric *Escherichia coli* isolated from aboriginal children from the Kimberley region of Western Australia. *J Infect Dis* **167**: 755–758.

Harrington SM, Strauman MC, Abe CM & Nataro JP (2005) Aggregative adherence fimbriae contribute to the inflammatory response of epithelial cells infected with enteroaggregative *Escherichia coli*. *Cell Microbiol* **7**: 1565–1578.

Hart A, Pham T, Nowicki S, Whorton EB Jr, Martens MG, Anderson GD & Nowicki BJ (1996) Gestational pyelonephritis-associated *Escherichia coli* isolates represent a nonrandom, closely related population. *Am J Obstet Gynecol* **174**: 983–989.

Hart A, Nowicki BJ, Reisner B, *et al.* (2001) Ampicillin-resistant *Escherichia coli* in gestational pyelonephritis: increased occurrence and association with the colonization factor Dr adhesin. *J Infect Dis* **183**: 1526–1529.

Hasan RJ, Pawelczyk E, Urvil PT, *et al.* (2002) Structure–function analysis of decay-accelerating factor: identification of residues important for binding of the *Escherichia coli* Dr adhesin and complement regulation. *Infect Immun* **70**: 4485–4493.

Hudault S, Spiller OB, Morgan BP & Servin AL (2004) Human diffusely adhering *Escherichia coli* expressing Afa/Dr adhesins that use human CD55 (decay-accelerating factor) as a receptor does not bind the rodent and pig analogues of CD55. *Infect Immun* **72**: 4859–4863.

Humbert J, Jouve M, Le Bouguenec C & Gounon P (2000) Electron microscopic improvement in the study of diarrheagenic *Escherichia coli*. *Microsc Res Tech* **49**: 383–393.

Ishitoya S, Yamamoto S, Kanamaru S, Kurazono H, Habuchi T, Ogawa O & Terai A (2003) Distribution of afaE adhesins in *Escherichia coli* isolated from Japanese patients with urinary tract infection. *J Urol* **169**: 1758–1761.

Johnson JR (2003) Microbial virulence determinants and the pathogenesis of urinary tract infection. *Infect Dis Clin North Am* **17**: 261–278.

Jouve M, Garcia MI, Courcoux P, Labigne A, Gounon P & Le Bouguenec C (1997) Adhesion to and invasion of HeLa cells by pathogenic *Escherichia coli* carrying the afa-3 gene cluster are mediated by the AfaE and AfaD proteins, respectively. *Infect Immun* **65**: 4082–4089.

Kansau I, Berger C, Hospital M, Amsellem R, Nicolas V, Servin AL & Bernet-Camard MF (2004) Zipper-like internalization of Dr-positive *Escherichia coli* by epithelial cells is preceded by an adhesin-induced mobilization of raft-associated molecules in the initial step of adhesion. *Infect Immun* **72**: 3733–3742.

Kaper JB, Nataro JP & Mobley HLT (2004) Pathogenic *Escherichia coli*. *Nat Rev Microbiol* **2**: 123–140.

Khan MA, Kang J & Steiner TS (2004) Enteroaggregative *Escherichia coli* flagellin-induced interleukin-8 secretion requires Toll-like receptor 5-dependent p38 MAP kinase activation. *Immunology* **112**: 651–660.

Labigne-Roussel AF, Lark D, Schoolnik G & Falkow S (1984) Cloning and expression of an afimbrial adhesin (Afa-I) responsible for P blood group-independent, mannose-resistant hemagglutination from a pyelonephritic *Escherichia coli* strain. *Infect Immun* **46**: 251–259.

Lalioui L & Le Bouguenec C (2001) afa-8 Gene cluster is carried by a pathogenicity island inserted into the tRNA(Phe) of human and bovine pathogenic *Escherichia coli* isolates. *Infect Immun* **69**: 937–948.

Lalioui L, Jouve M, Gounon P & Le Bouguenec C (1999) Molecular cloning and characterization of the afa-7 and afa-8 gene clusters encoding afimbrial adhesins in *Escherichia coli* strains associated with diarrhea or septicemia in calves. *Infect Immun* **67**: 5048–5059.

Le Bouguenec C (1999) Diarrhea-associated diffusely adherent *Escherichia coli*. *Clin Microbiol Rev* **12**: 180–181.

Le Bouguenec C, Archambaud M & Labigne A (1992) Rapid and specific detection of the pap, afa, and sfa adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. *J Clin Microbiol* **30**: 1189–1193.

Le Bouguenec C, Garcia MI, Ouin V, Desperrier JM, Gounon P & Labigne A (1993) Characterization of plasmid-borne afa-3 gene clusters encoding afimbrial adhesins expressed by *Escherichia coli* strains associated with intestinal or urinary tract infections. *Infect Immun* **61**: 5106–5114.

Le Bouguenec C, Lalioui L, du Merle L, *et al.* (2001) Characterization of AfaE adhesins produced by extraintestinal and intestinal human *Escherichia coli* isolates: PCR assays for detection of Afa adhesins that do or do not recognize Dr blood group antigens. *J Clin Microbiol* **39**: 1738–1745.

Levine MM, Ferreccio C, Prado V, *et al.* (1993) Epidemiologic studies of *Escherichia coli* diarrheal infections in a low socioeconomic level peri-urban community in Santiago, Chile. *Am J Epidemiol* **138**: 849–869.

Lublin DM, Kompelli S, Storry JR & Reid ME (2000) Molecular basis of Cromer blood group antigens. *Transfusion* **40**: 208–213.

Nataro JP & Kaper JB (1998) Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* **11**: 142–201.

Nowicki B, Barrish JP, Korhonen T, Hull RA & Hull SI (1987) Molecular cloning of the *Escherichia coli* O75X adhesin. *Infect Immun* **55**: 3168–3173.

Nowicki B, Svanborg-Eden C, Hull R & Hull S (1989) Molecular analysis and epidemiology of the Dr hemagglutinin of uropathogenic *Escherichia coli*. *Infect Immun* **57**: 446–451.

Nowicki B, Labigne A, Moseley S, Hull R, Hull S & Moulds J (1990) The Dr hemagglutinin, afimbrial adhesins Afa-I and Afa-III, and F1845 fimbriae of uropathogenic and diarrhea-associated *Escherichia coli* belong to a family of hemagglutinins with Dr receptor recognition. *Infect Immun* **58**: 279–281.

Nowicki B, Hart A, Coyne KE, Lublin DM & Nowicki S (1993) Short consensus repeat-3 domain of recombinant decay-accelerating factor is recognized by *Escherichia coli* recombinant Dr adhesin in a model of a cell–cell interaction. *J Exp Med* **178**: 2115–2121.

Nowicki B, Martens M, Hart A & Nowicki S (1994) Gestational age-dependent distribution of *Escherichia coli* fimbriae in pregnant patients with pyelonephritis. *Ann N Y Acad Sci* **730**: 290–291.

Nowicki B, Selvarangan R & Nowicki S (2001) Family of *Escherichia coli* Dr adhesins: decay-accelerating factor receptor recognition and invasiveness. *J Infect Dis* **183**(Suppl 1): S24–S27.

Oelschlaeger TA, Dobrindt U & Hacker J (2002) Virulence factors of uropathogens. *Curr Opin Urol* **12**: 33–38.

Orchard TR, Dhar A, Simmons JD, Vaughan R, Welsh KI & Jewell DP (2001) MHC class I chain-like gene A (MICA) and its associations with inflammatory bowel disease and peripheral arthropathy. *Clin Exp Immunol* **126**: 437–440.

Peiffer I, Servin AL & Bernet-Camard MF (1998) Piracy of decay-accelerating factor (CD55) signal transduction by the diffusely adhering strain *Escherichia coli* C1845 promotes cytoskeletal F-actin rearrangements in cultured human intestinal INT407 cells. *Infect Immun* **66**: 4036–4042.

Peiffer I, Blanc-Potard AB, Bernet-Camard MF, Guignot J, Barbat A & Servin AL (2000a) Afa/Dr diffusely adhering *Escherichia coli* C1845 infection promotes selective injuries in the

junctional domain of polarized human intestinal Caco-2/TC7 cells. *Infect Immun* **68**: 3431–3442.

Peiffer I, Guignot J, Barbat A, *et al.* (2000b) Structural and functional lesions in brush border of human polarized intestinal Caco-2/TC7 cells infected by members of the Afa/Dr diffusely adhering family of *Escherichia coli*. *Infect Immun* **68**: 5979–5990.

Peiffer I, Bernet-Camard MF, Rousset M & Servin AL (2001) Impairments in enzyme activity and biosynthesis of brush border- associated hydrolases in human intestinal Caco-2/TC7 cells infected by members of the Afa/Dr family of diffusely adhering *Escherichia coli*. *Cell Microbiol* **3**: 341–357.

Pettigrew D, Anderson KL, Billington J, *et al.* (2004) High resolution studies of the Afa/Dr adhesin DraE and its interaction with chloramphenicol. *J Biol Chem* **279**: 46851–46857.

Pham TQ, Goluszko P, Popov V, Nowicki S & Nowicki BJ (1997) Molecular cloning and characterization of Dr-II, a nonfimbrial adhesin- I-like adhesin isolated from gestational pyelonephritis-associated *Escherichia coli* that binds to decay-accelerating factor. *Infect Immun* **65**: 4309–4318.

Piatek R, Zalewska B, Kolaj O, Ferens M, Nowicki B & Kur J (2005) Molecular aspects of biogenesis of *Escherichia coli* Dr fimbriae: characterization of DraB–DraE complexes. *Infect Immun* **73**: 135–145.

Plancon L, Du Merle L, Le Friec S, *et al.* (2003) Recognition of the cellular beta1-chain integrin by the bacterial AfaD invasin is implicated in the internalization of afa-expressing pathogenic *Escherichia coli* strains. *Cell Microbiol* **5**: 681–693.

Sauer FG, Futterer K, Pinkner JS, Dodson KW, Hultgren SJ & Waksman G (1999) Structural basis of chaperone function and pilus biogenesis. *Science* **285**: 1058–1061.

Scaletsky IC, Fabbricotti SH, Carvalho RL, Nunes CR, Maranhao HS, Morais MB & Fagundes-Neto U (2002) Diffusely adherent *Escherichia coli* as a cause of acute diarrhea in young children in Northeast Brazil: a case–control study. *J Clin Microbiol* **40**: 645–648.

Selvarangan R, Goluszko P, Popov V, *et al.* (2000) Role of decay-accelerating factor domains and anchorage in internalization of Dr-fimbriated *Escherichia coli*. *Infect Immun* **68**: 1391–1399.

Selvarangan R, Goluszko P, Singhal J, *et al.* (2004) Interaction of Dr adhesin with collagen type IV is a critical step in *Escherichia coli* renal persistence. *Infect Immun* **72**: 4827–4835.

Servin AL (2005) Pathogenicity of Afa/Dr diffusely adhering *Escherichia coli*. *Clin Microbiol Rev* **18**: 264–292.

Soto GE & Hultgren SJ (1999) Bacterial adhesins: common themes and variations in architecture and assembly. *J Bacteriol* **181**: 1059–1071.

Taddei CR, Moreno AC, Fernandes Filho A, Montemor LP & Martinez MB (2003) Prevalence of secreted autotransporter toxin gene among diffusely adhering *Escherichia coli* isolated from stools of children. *FEMS Microbiol Lett* **227**: 249–253.

Taddei CR, Fasano A, Ferreira AJ, Trabulsi LR & Martinez MB (2005) Secreted autotransporter toxin produced by a diffusely adhering *Escherichia coli* strain causes intestinal damage in animal model assays. *FEMS Microbiol Lett* **250**: 263–269.

Tieng V, Le Bouguenec C, du Merle L, *et al.* (2002) Binding of *Escherichia coli* adhesin AfaE to CD55 triggers cell-surface expression of the MHC class I-related molecule MICA. *Proc Natl Acad Sci USA* **99**: 2977–2982.

Usein CR, Damian M, Tatu-Chitoiu D, *et al.* (2001) Prevalence of virulence genes in *Escherichia coli* strains isolated from Romanian adult urinary tract infection cases. *J Cell Mol Med* **5**: 303–310.

Van Loy CP, Sokurenko EV & Moseley SL (2002) The major structural subunits of Dr and F1845 fimbriae are adhesins. *Infect Immun* **70**: 1694–1702.

Westerlund B, Kuusela P, Risteli J, *et al.* (1989) The O75X adhesin of uropathogenic *Escherichia coli* is a type IV collagen-binding protein. *Mol Microbiol* **3**: 329–337.

Wroblewska-Seniuk K, Selvarangan R, Hart A, *et al.* (2005) Dra/AfaE adhesin of uropathogenic Dr/Afa+ *Escherichia coli* mediates mortality in pregnant rats. *Infect Immun* **73**: 7597–7601.

Zalewska B, Piatek R, Cieslinski H, Nowicki B & Kur J (2001) Cloning, expression, and purification of the uropathogenic *Escherichia coli* invasin DraD. *Protein Expr Purif* **23**: 476–482.

Zalewska B, Piatek R, Bury K, Samet A, Nowicki B, Nowicki S & Kur J (2005) A surface-exposed DraD protein of uropathogenic *Escherichia coli* bearing Dr fimbriae may be expressed and secreted independently from DraC usher and DraE adhesin. *Microbiology* **151**: 2477–2486.

Zhang L & Foxman B (2003) Molecular epidemiology of *Escherichia coli* mediated urinary tract infections. *Front Biosci* **8**: e235–e244.

Zhang L, Foxman B, Tallman P, Cladera E, Le Bouguenec C & Marrs CF (1997) Distribution of drb genes coding for Dr binding adhesins among uropathogenic and fecal *Escherichia coli* isolates and identification of new subtypes. *Infect Immun* **65**: 2011–2018.

Zhou X, Giron JA, Torres AG, Crawford JA, Negrete E, Vogel SN & Kaper JB (2003) Flagellin of enteropathogenic *Escherichia coli* stimulates interleukin-8 production in T84 cells. *Infect Immun* **71**: 2120–2129.