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Binding of the Type 3 Fimbriae of *Klebsiella pneumoniae* to Human Endothelial and Urinary Bladder Cells

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Binding of the two identified type 3 fimbrial variants of *Klebsiella pneumoniae* to human endothelial EA-hy926 and bladder T24 cells was assessed. The recombinant *Escherichia coli* strain LE392(pFK12), expressing plasmid-encoded type 3 fimbriae of *K. pneumoniae*, adhered to both cell lines, and the fimbriae purified from the strain bound to both cell lines in a dose-dependent manner. Adhesiveness to both cell lines of chromosomally encoded type 3 fimbriae from *K. pneumoniae* IApc35 was lower. No binding was detected with type 1 fimbriae of *K. pneumoniae*. Both type 3 fimbrial variants exhibited a significantly lower affinity for the cell lines than did S fimbriae of meningitis-associated *E. coli*.

Klebsiella pneumoniae and *Klebsiella oxytoca* are opportunistic pathogens associated with complicated urinary tract infections, nosocomial pneumonia, infections of surgical wounds, and septic infections. Urinary tract infections by *Klebsiella* species are commonly encountered in patients who have predisposing factors such as indwelling catheters or primary infections by other microorganisms (16, 22, 33). These infections often occur in hospitalized or intensive care unit patients and frequently lead to sepsis as well as chronic or recurrent urinary tract infections (1, 16, 28, 33). Bacterial factors proposed to contribute to *Klebsiella* virulence include adhesive fimbriae, iron scavenging systems, production of urease, and capsular antigens (7, 11, 17, 18, 20, 26, 28, 30). Many of the *Klebsiella* isolates from patients are resistant to a number of antibiotics (29).

Four types of adhesins have been detected on *Klebsiella* strains. Type 1 fimbriae are characterized by their binding to mannosides, and type 3 fimbriae are characterized by their capacity to react with tannin-treated erythrocytes (5). A fimbrial antigen termed KPF-28 was recently described in β-lactamase-producing isolates of *K. pneumoniae* (4). A nonfimbrial adhesin termed CF29K is encoded by a resistance plasmid and has been indicated to be involved in the adherence of *K. pneumoniae* to human intestinal cells (2, 3). Type 3 fimbriae are expressed by most, if not all, uropathogenic or nosocomial isolates of *Klebsiella* (17, 26, 30). The functional expression of type 3 fimbriae of *K. pneumoniae* requires six *mrk* genes, of which *mrkA* encodes the major fimbrial subunit and *mrkD* encodes the adhesin subunit (8, 9). Recent reports (13, 27) have demonstrated that type 3 fimbriae of *K. pneumoniae* can occur as two variants, a plasmid-encoded variant and a chromosomally encoded variant which differ in the *mrkD* adhesin gene. We have presented evidence that the plasmid-borne variant of type 3 fimbriae of *K. pneumoniae* binds to basolateral aspects of renal tubular cells (29). Hornick et al. (12) similarly demonstrated that this type 3 fimbrial variant binds to the basolateral surface of tracheal cells and to the basal epithelial cells and the basement membrane regions of bronchial epithelia. We have identified type V collagen, a component of extracellular matrices, as the renal target for this variant of type 3 fimbriae (29). The chromosome-encoded variant of *K. pneumoniae* type 3 fimbriae does not hemagglutinate and bind to type V collagen (13, 27), and its binding properties have remained uncharacterized. An isolate of *K. pneumoniae* can express both type 3 fimbrial variants (13, 27).

The results cited above indicate that the type 3 fimbriae of *K. pneumoniae* mediate bacterial adherence to basolateral aspects of human urinary tract and respiratory tissue. On the other hand, the role of type 3 fimbriae in adhesion of *Klebsiella* to human epithelial cells has remained open. We have presented evidence that plasmid-encoded type 3 fimbriae bind to sediment cells from normal human urine (30), whereas Podschun et al. (25) found no correlation between the expression of type 3 fimbriae and bacterial adhesiveness to cultured HeLa and Intestine 407 cells and concluded that fimbriae are not involved in the binding of *Klebsiella* cells to eucaryotic cells. As the capacity of type 3 fimbriae to bind to epithelial cells has remained controversial, we undertook the present study to evaluate their binding to cultured human endothelial and urinary bladder cells. Furthermore, we compared the adhesiveness of the two type 3 fimbrial variants to that shown by the sialic acid-binding S fimbria of meningitis-associated *Escherichia coli*, as its adhesiveness to endothelial and bladder cells has been well described (23, 24, 30).

The plasmids and *K. pneumoniae* strains used in this study are shown in Table 1. The fimbria-encoding plasmids were transformed by standard procedures to the nonfimbriate *E. coli* strain LE392, which was used as the host in the adherence assays. Bacteria were grown overnight on Luria plates supplemented with antibiotics, collected, and suspended to a density of 10^{10} cells/ml in phosphate-buffered saline, pH 7.1 (PBS). Before use in adherence tests, the proper expression of fimbriae by the strains was assessed by hemagglutination, mannose-sensitive yeast cell agglutination, and agglutination in fimbria-specific antisera by standard procedures (5, 14, 15, 30). Human endothelial EA-hy926 cells (HEC) (6) were cultured to confluence in RPMI 1640 medium (Life Technologies Gibco BRL, Paisley, Scotland) supplemented with 10% (vol/vol) fetal calf serum (PAA Laboratories GmbH, Linz, Austria), 2 mM L-glutamine (Life Technologies), penicillin (100 IU/ml), and streptomycin (100 μg/ml). Human bladder transitional T24 cells (ATCC HTB-4) were cultured in McCoy's 5a medium (Life Technologies) supplemented with fetal calf serum, L-glutamine, penicillin, and streptomycin as described

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TABLE 1. Plasmids and *K. pneumoniae* strains used in this study

Plasmid or strain	Relevant geno- or phenotype	Reference or source
pANN-801-13	*sfaABCDEFGHS* of *E. coli*	10
pANN801-118	SfaS with the change Arg-118 to Ser-118	19
pFK12	*mrkABCD* from *K. pneumoniae* IA565	8, 9
pGG101	*fimABCDEGHIJ* from *K. pneumoniae* IA565	9
K. pneumoniae IApc35	Plasmid-cured derivative of IA565	13
K. pneumoniae IAΔT3	IApc35 with *mrkB*::Tn*5*	D. Hornick

above. The cell lines were cultured on diagnostic glass slides (Knittel Glassbearbeitungs GmbH, Braunschweig, Germany) for adherence tests and on 96-well tissue culture plates (Nunc, Roskilde, Denmark) for binding tests. In adherence tests, the epithelial cells on glass slides were washed for 5 min with PBS and then fixed for 10 min at 4°C in 3.5% (wt/vol) paraformaldehyde in PBS (31). After three washes with PBS, 40 μl of bacterial suspension was added and the slides were incubated for 2 h at 4°C (HEC) or 37°C (T24 cells). The slides were then washed three times with PBS, stained for 10 min with 10% (wt/vol) Giemsa stain (Reagena Ltd, Kuopio, Finland), and examined with a BX50 microscope (Olympus Optical Co., Hamburg, Germany). The mean number and standard deviation of adherent bacteria on 50 epithelial cells were calculated. In the case of *E. coli* LE392(pGG101), the adherence tests were also performed in the presence of 25 mM α-methyl mannoside. The strains were tested five times; the results shown in Fig. 1 are from a representative assay with two independent parallels.

We included in the assays *E. coli* LE392(pANN801-13) as a positive control and *E. coli* LE392(pANN801-118) with a nonfunctional SfaS lectin as a negative control (Fig. 1). *E. coli* LE392(pFK12) adhered to both cell lines significantly more efficiently than did the nonfimbriate strain *E. coli* LE392 (Fig. 1) and *E. coli* LE392(pACYC184) containing the vector plasmid only (data not shown). *E. coli* LE392(pGG101) did not exhibit significant adhesiveness, and α-methyl-D-mannoside had no effect on the adherence. *K. pneumoniae* IApc35, carrying the chromosomally encoded type 3 fimbriae, showed an adhesion level that was higher than that shown by nonfimbriate *K. pneumoniae* IAΔT3 but lower than the one shown by *E. coli* LE392(pFK12) with the plasmid-encoded type 3 fimbriae.

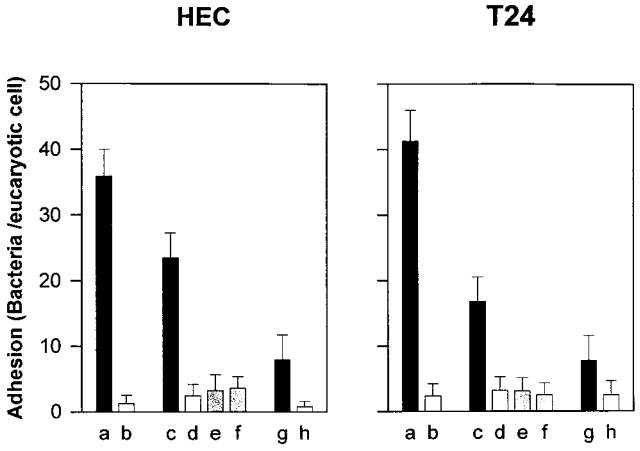

FIG. 1. Adhesion of bacteria to human endothelial cells (HEC) and human bladder cells (T24). Bars: a, S-fimbriate *E. coli* LE392(pANN801-13); b, *sfaS*-mutant LE392(pANN801-118); c, type 3-fimbriate *E. coli* LE392(pFK12); d, nonfimbriate *E. coli* LE392; bar e, type 1-fimbriate *E. coli* LE392(pGG101); f, *E. coli* LE392(pGG101) tested in the presence of 25 mM α-methyl mannoside; g, type 3-fimbriate *K. pneumoniae* IApc35; h, nonfimbriate *K. pneumoniae* IAΔT3. The mean numbers and standard deviations of bacteria adhering to 50 eucaryotic cells are shown.

Adherence to HEC and T24 cells is shown in Fig. 2. Whereas *E. coli* LE392(pFK12) and *E. coli* LE392(pANN801-13) (not shown) adhered evenly along the epithelial cell surfaces, we observed that strain IApc35 often adhered in clusters along the edges of the epithelial cells (Fig. 2).

To confirm the role of fimbriae in bacterial adherence to HEC and T24 cells, we assessed the fimbriae purified from *E. coli* LE392(pANN801-13), *E. coli* LE392(pANN801-118), *E. coli* LE392(pGG101), *E. coli* LE392(pFK12), and *K. pneumoniae* IApc35 for binding to the cell lines. The fimbriae were purified by using deoxycholate and concentrated urea (15), and the antibodies used in detection of the bound fimbriae have been described earlier (29, 30). Prior to the assays, we absorbed the antisera with HEC cells to remove any cross-reactivity with the epithelial cells. We used the antiserum raised against purified type 3 fimbriae from *E. coli* HB101(pFK12) to detect both variants of type 3 fimbriae. An enzyme-linked immunosorbent assay with purified fimbriae from LE392(pFK12) and IApc35 as antigens (15) revealed no significant difference in their reactivities with the antiserum (data not shown). The binding of the fimbriae to the epithelial cells was tested essentially as described earlier (24). Briefly, HEC and the T24 cells on microtiter plate wells were washed three times for 5 min each time in Dulbecco PBS (Nord Cell, Skärholmen, Sweden) containing 0.1% (wt/vol) bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Mo.), and the fimbriae (1 to 100 μg/ml in 100 μl of Dulbecco PBS-BSA) were incubated on the cells for 5 h at 4°C. The plates were washed three times for 5 min each time at 4°C with Dulbecco PBS-BSA and then fixed for 10 min at 4°C with 3.5% (wt/vol) paraformaldehyde in Dulbecco PBS-BSA. After the plates were washed three times with PBS-BSA, 100 μl of the antifimbria antiserum (diluted 1/1,000 in PBS-BSA) was added and the plates were incubated overnight at 4°C. After three washes, the bound antibodies were detected with alkaline phosphatase-conjugated anti-rabbit immunoglobulin G (Orion Diagnostica, Espoo, Finland) by using *p*-nitrophenyl phosphate as a substrate. The binding tests were performed five times; the results in Fig. 3 are given as mean values from a representative assay with two independent parallels.

The specificity of the binding assay was indicated by the efficient binding to both cell types of the purified S fimbriae and by the lack of binding of the *sfaS* mutant fimbriae (Fig. 3). The fimbriae purified from *E. coli* LE392(pFK12) exhibited a moderate binding, whereas the binding by type 1 fimbriae of *K. pneumoniae* to both cell types was similar to that shown by the LE392(pANN801-118) fimbriae. With HEC, the adhesiveness of the IApc35 fimbriae was lower than that of the LE392(pFK12) fimbriae but higher than that shown by the LE392(pGG101) fimbriae, and with T24 cells, the adhesiveness was close to that seen with the negative control fimbriae (Fig. 3).

Our results demonstrate that the type 3 fimbriae of *K. pneumoniae* have the capacity to bind to human endothelial and urinary bladder cells. The binding of type 3 fimbriae was demonstrated by two lines of experimental design. Recombinant *E. coli* expressing plasmid-borne fimbriae and *K. pneumoniae* IApc35 with the chromosomal variant bound to both cell types with a higher affinity than did the isogenic nonfimbriate strains. Second, we demonstrate binding of purified type 3 fimbriae to both cell lines. In contrast, we did not detect any binding of

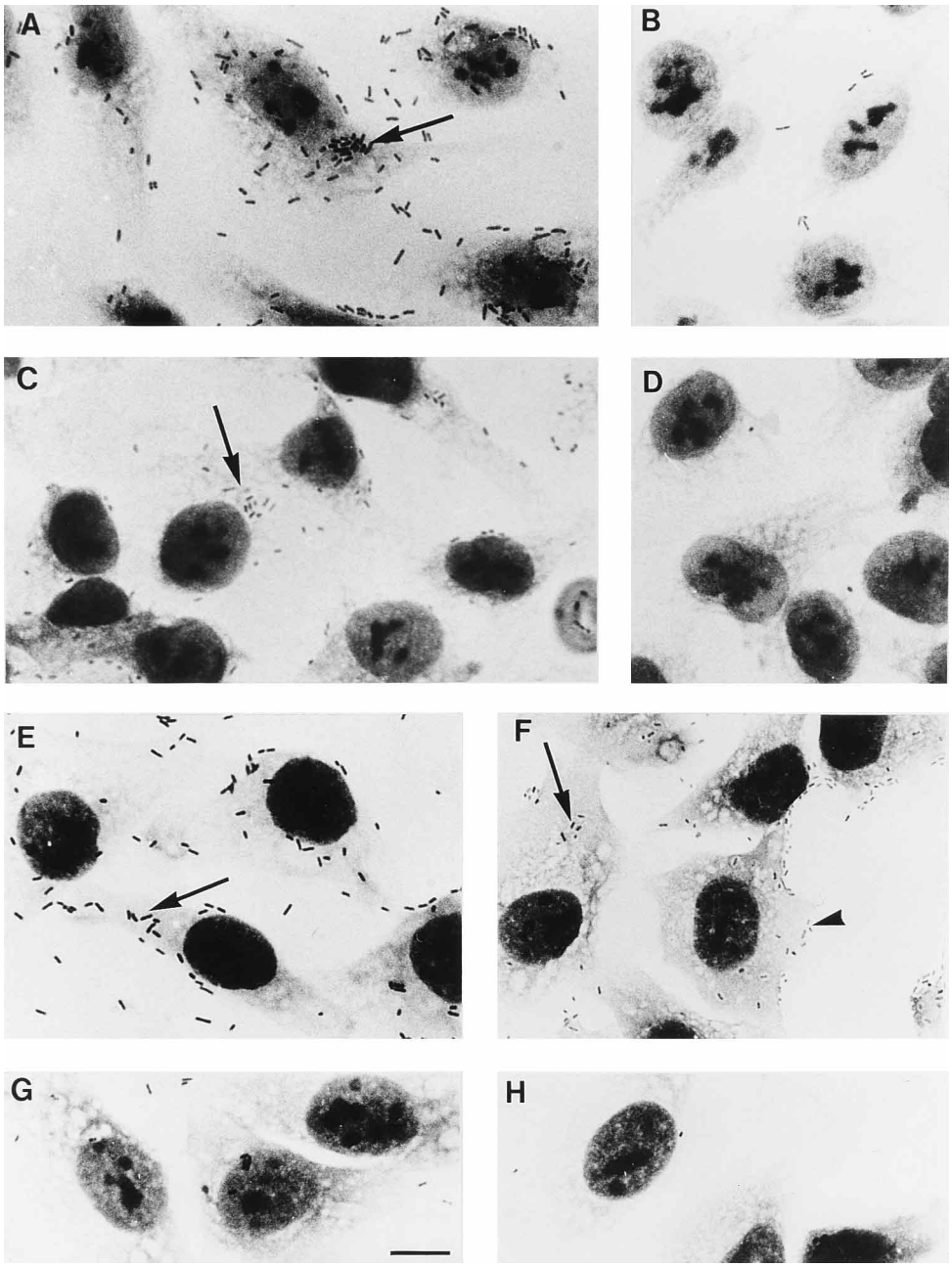

FIG. 2. Light microscopy of bacterial adherence to human endothelial cells (A to D) and human bladder cells (E to H). The strains are type 3-fimbriate *E. coli* LE392(pFK12) (A and E), nonfimbriate *E. coli* LE392 (B and G), type 3-fimbriate *K. pneumoniae* IApc35 (C and F), and nonfimbriate *K. pneumoniae* IAΔT3 (D and H). Arrows indicate bacterial adherence to epithelial cells, and the arrowhead in panel F indicates adhesion of strain IApc35 to the edges of the T24 bladder cells. Size bar, 10 μm.

type 1 fimbriae. Our results also indicate that the two type 3 fimbrial variants differ in their affinities for eucaryotic cells. The plasmid-encoded variant bound with a higher affinity to the eucaryotic cells. The physical basis of this difference remains to be established; at present it is known that the two variants differ in the *mrkD* gene encoding the minor adhesin protein of the filament (13, 27).

The binding of type 3 fimbriae to human endothelial cells may contribute to establishment of urosepsis by klebsiellas and to induction of endothelial responses against lipopolysaccharide, for example. Klebsiellas are associated with urinary tract infections in the elderly or in compromised patients suffering from predisposing factors such as tissue trauma. The high affinity of type 3 fimbriae for type V collagen (29) is compatible with infections at damaged tissue sites, as type V collagen molecules are not accessible at intact luminal epithelial surfaces. Our results indicate that type 3 fimbriae also express affinity for bladder epithelial cells; their binding, however, is of low affinity compared to that shown by S fimbriae of *E. coli*. The low affinity of type 3 fimbriae for uroepithelium probably contributes to the poor infectivity of klebsiellas in a normal urinary tract. The binding properties of plasmid-encoded type

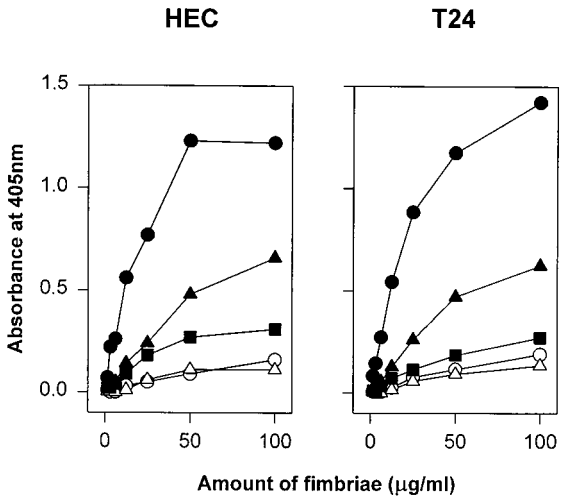

FIG. 3. Binding of purified fimbriae to human endothelial cells (HEC) and human bladder cells (T24). Symbols: ●, S fimbriae from *E. coli* LE392 (pANN801-13); ▲, type 3 fimbriae from *E. coli* LE392(pFK12); ■, type 3 fimbriae from *K. pneumoniae* IApc35; ○, SfaS-mutant fimbriae from *E. coli* LE392 (pANN801-118); △, type 1 fimbriae from *E. coli* LE392(pGG101).

3 fimbriae are similar to those of Dr fimbriae associated with lower urinary tract infections. In the kidney, Dr fimbriae bind to type IV collagen of basement membranes and are strictly nonepithelial in their binding (reviewed in reference 32). Dr fimbriae, however, bind to decay-accelarating factor of epithelial cells in the lower urinary tract (21). The binding of Dr fimbriae to both receptors apparently involves a highly similar receptor-active domain on the target proteins. We have earlier shown that normal human urine does not contain secreted inhibitors of type 3 fimbrial binding (30), which suggests that type 3 fimbriae function in vivo in the human urinary tract. The binding of type 3 fimbriae to urinary bladder cells, although of low affinity, may contribute to bacterial colonization of uroepithelia in patients with impaired renal clearence and defense mechanisms.

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