JOURNAL OF CLINICAL MICROBIOLOGY, June 2010, p. 2316–2317 Vol. 48, No. 6
0095-1137/10/$12.00 doi:10.1128/JCM.00611-10

Ag43 Promotes Persistence of Uropathogenic *Escherichia coli* Isolates in the Urinary Tract[▽]

Uropathogenic *Escherichia coli* (UPEC) harbors an arsenal of virulence factors which enable it to infect the host. Adhesion factors of *E. coli*, facilitating the initial colonization of epithelia and eventually leading to invasion of the host cell, have been described extensively elsewhere (2, 3). More recently, factors supporting *E. coli* persistence have also attracted increasing interest (6, 7, 11).

Variants of the autotransporter protein Ag43 are expressed by pathogenic as well as commensal *E. coli* strains (10). By using a mouse infection model, differential functions during pathogenesis were revealed for the two Ag43 proteins in UPEC strain CFT073, encoded by the CFT073 *fluA* gene ($fluA_{CFT073}$) and $fluB_{CFT073}$ (9). Whereas Ag43a displayed higher-level autoaggregative properties and promoted persistence in the urinary bladder, Ag43b impaired initial colonization but did not affect later stages of infection. We here investigated clinical UPEC isolates from sporadic versus recurrent urinary tract infections (UTI) to demonstrate the relevance of this observation for human tissue.

A total of 118 *E. coli* isolates were collected from 47 children with sporadic and recurrent UTI (5). Biochemical differentiation and macrorestriction analyses identified 85 different *E. coli* strains; 12 strains were repeatedly recovered from the same patients at visits at least 1 month apart (recurrent strains), whereas the remaining strains were isolated only once (sporadic strains). All isolates were tested for the presence of *flu* (9) and the two CFT073-specific gene variants $fluA_{CFT073}$ and $fluB_{CFT073}$ (8).

The *flu* gene was detected in 55 of 85 isolates (65%), and we observed a significant association with isolates from recurrent infections (11 [92%] of 12 such isolates had the gene, versus 44 [60%] of 73 isolates from sporadic infections [$P < 0.05$]). The overall prevalence for $fluA_{CFT073}$ and $fluB_{CFT073}$ was 27% each (each gene was present in 23 of 85 isolates). Although neither gene was linked to recurrent strains, the common presence of both genes in one strain was more often found in recurrent than in sporadic strains of the tested collection (5 of 12 recurrent strains versus 10 of 73 sporadic strains [$P < 0.05$]). Thus, our data support the previously reported linkage between Ag43 proteins and intracellular persistence (1). We further sought for characteristics which could possibly explain the differential roles of $fluA_{CFT073}$ and $fluB_{CFT073}$ observed during mouse UTI (9).

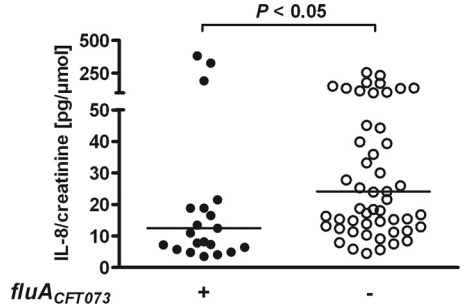

FIG. 1. IL-8 levels in urine from children with UTI caused by *E. coli* strains, in relation to the presence ($n = 23$) or absence ($n = 57$) of $fluA_{CFT073}$. Individual levels and medians are presented. The difference is significant (by the Mann-Whitney U test).

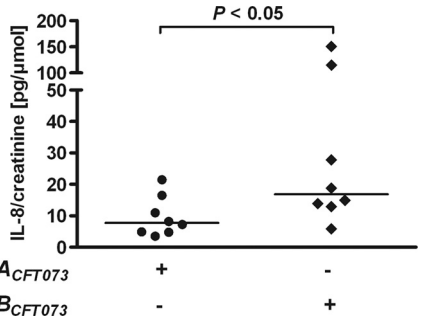

FIG. 2. IL-8 levels in urine from children with UTI caused by *E. coli* strains carrying only $fluA_{CFT073}$ ($n = 8$) or only $fluB_{CFT073}$ ($n = 8$). Individual levels and medians are presented. The difference is significant (by the Mann-Whitney U test).

The neutrophil-attractant chemokine interleukin-8 (IL-8) is assigned a crucial role in bacterial clearance from the urinary tract (4). The IL-8/creatinine ratios in urine samples from children infected with $fluA_{CFT073}$-positive strains were significantly lower than those in samples from children infected with strains lacking this gene ($P < 0.05$) (Fig. 1), and isolates carrying only $fluA_{CFT073}$ were associated with lower IL-8 levels than isolates expressing only $fluB_{CFT073}$ ($P < 0.05$) (Fig. 2). These findings suggest that $fluB_{CFT073}$ mediates a more immunogenic bacterial phenotype, which could explain the previously reported interference of Ag43b with early stages of infection (9). In contrast, a higher degree of bacterial autoaggregation in strains expressing $fluA_{CFT073}$ (9) might reduce the exposure of immunogenic surface structures, thereby allowing evasion of immune recognition and longer bacterial persistence in the host. Taken together, clinical data from the UPEC collection investigated here support the suggested roles of Ag43 variants in type strain CFT073 and underline the impact of Ag43 proteins on bacterial persistence in the urinary tract. They further indicate that both $fluA_{CFT073}$ and $fluB_{CFT073}$ might be of advantage for UPEC.

We thank Tiina Tallvik for help collecting the *E. coli* isolates.

This work was supported by grants from the Swedish Research Council (no. 56X-20356), ALF Project Funding, and the Karolinska Institutet.

REFERENCES

1. **Anderson, G. G., J. J. Palermo, J. D. Schilling, R. Roth, J. Heuser, and S. J. Hultgren.** 2003. Intracellular bacterial biofilm-like pods in urinary tract infections. Science **301:**105–107.
2. **Antão, E.-M., L. Wieler, and C. Ewers.** 2009. Adhesive threads of extraintestinal pathogenic *Escherichia coli*. Gut Pathog. **1:**22.
3. **Fronzes, R., H. Remaut, and G. Waksman.** 2008. Architectures and biogenesis of non-flagellar protein appendages in Gram-negative bacteria. EMBO J. **27:**2271–2280.
4. **Godaly, G., A. E. Proudfoot, R. E. Offord, C. Svanborg, and W. W. Agace.** 1997. Role of epithelial interleukin-8 (IL-8) and neutrophil IL-8 receptor A in *Escherichia coli*-induced transuroepithelial neutrophil migration. Infect. Immun. **65:**3451–3456.

5. **Hertting, O., M. Chromek, Z. Slamová, L. Kádas, M. Söderkvist, I. Vainumäe, T. Tallvik, S. H. Jacobson, and A. Brauner.** 2008. Cytotoxic necrotizing factor 1 (CNF1) induces an inflammatory response in the urinary tract *in vitro* but not *in vivo*. Toxicon **51:**1544–1547.
6. **Mysorekar, I. U., and S. J. Hultgren.** 2006. Mechanisms of uropathogenic *Escherichia coli* persistence and eradication from the urinary tract. Proc. Natl. Acad. Sci. U. S. A. **103:**14170–14175.
7. **Nicholson, T. F., K. M. Watts, and D. A. Hunstad.** 2009. OmpA of uropathogenic *Escherichia coli* promotes postinvasion pathogenesis of cystitis. Infect. Immun. **77:**5245–5251.
8. **Restieri, C., G. Garriss, M.-C. Locas, and C. M. Dozois.** 2007. Autotransporter-encoding sequences are phylogenetically distributed among *Escherichia coli* clinical isolates and reference strains. Appl. Environ. Microbiol. **73:**1553–1562.
9. **Ulett, G. C., J. Valle, C. Beloin, O. Sherlock, J.-M. Ghigo, and M. A. Schembri.** 2007. Functional analysis of antigen 43 in uropathogenic *Escherichia coli* reveals a role in long-term persistence in the urinary tract. Infect. Immun. **75:**3233–3244.
10. **van der Woude, M. W., and I. R. Henderson.** 2008. Regulation and function of Ag43 (flu). Annu. Rev. Microbiol. **62:**153–169.
11. **Wiles, T. J., R. R. Kulesus, and M. A. Mulvey.** 2008. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. Exp. Mol. Pathol. **85:**11.

Petra Lüthje
Annelie Brauner*
Clinical Microbiology
Department of Microbiology, Tumor and Cell Biology
Karolinska University Hospital and Karolinska Institutet
171 76 Stockholm, Sweden

*Phone: 46 8 5177 3914
Fax: 46 8 308099
E-mail: Annelie.Brauner@ki.se

▽ Published ahead of print on 31 March 2010.