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Short Communication

Role of S- and common-type I-fimbriae of *Escherichia coli* in experimental upper and lower urinary tract infection

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Introduction

Fimbrial antigens are very often associated with pathogenic *Escherichia coli* strains, especially those which cause extraintestinal infections.[1] These fimbriae have the ability to agglutinate erythrocytes (hemagglutination) and can be further grouped according to their receptor specificity. P-fimbriae recognize the 2-*D*-Gal-(1-4)-β-*D*-Gal region of globotetraosylceramide and trihexosylceramide, which are antigens of the human blood group P system. P-fimbriated *E. coli* are predominant in upper urinary tract infections (UTI) in man.[2] In contrast, S-fimbriae recognize a sialic acid-containing structure as receptor and are often associated with neonatal meningitis and, to a lesser extent, with UTI.[3,4] Mannose-sensitive hemagglutination fimbriae (Pili, also termed common type I fimbriae) use a mannose-containing receptor and are therefore inhibited in their hemagglutination capacity by the presence of mannose. These fimbriae are widespread among *E. coli* from different sources.[4,5]

Most pathogenic *E. coli* strains express more than one fimbrial type on their cell surface[6,7] (Boulnois & Jann, personal communication). Therefore it is hard to define the exact role of one single adhesin factor in infectious disease. In order to overcome this problem we have cloned the genetic determinants fort the S-fimbrial adhesin (*sfa*)[8] and for common type I fimbriae (*pil*) (Hacker *et al.*, in preparation) and introduced the recombinant DNAs in a non-fimbriated *E. coli* 06 strain. The two isogenic *E. coli* strains, which only differed in their fimbrial type and in an antibiotic recognition marker were tested in a rat UTI model, which has been shown fo fulfill the criteria for a persistent infection.[9]

Results and discussion

Figure 1 shows that in the kidney the number of Sfa$^+$ *E. coli* usually was higher than that of Pil$^+$ (common type I) *E. coli*. In the urinary bladder, however, the number of Pil$^+$ *E. coli* mostly exceeded that of the Sfa$^+$ strain. In the bladder the mean number of *E. coli* Pil$^+$ was 25 times *higher* than that of *E. coli* Sfa$^+$, whereas in the kidney the bacterial concentration of *E. coli* Pil$^+$ was 6 times *lower* than that of *E. coli* Sfa$^+$ (Table

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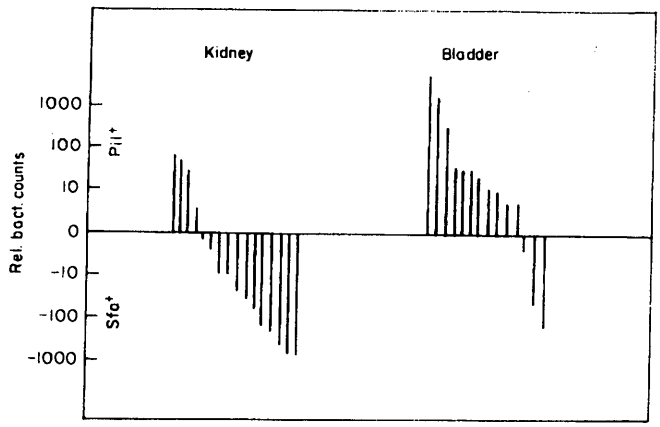

Fig. 1. The relative number of bacterial counts (difference between the concentration of Pil$^+$ *E. coli* and Sfa$^-$ *E. coli*) one week after infection with equal numbers of isogenic *E. coli* strains with Pil$^+$, Sfa$^-$ and Pil$^-$, Sfa$^+$ phenotype, respectively. Each column represents the relative bacterial counts of the bladder or both kidneys of one animal, i.e. the difference between the absolute number of bacteria in both organs. Positive values show the number of counts by which the Pil$^+$ *E. coli* exceed that of Sfa$^+$ *E. coli*. Negative values, in contrast, indicate the number of Sfa$^+$ *E. coli* in excess of Pil$^+$ *E. coli*.

Table 1 Bacterial counts (mean cfu/g tissue, standard deviation) in kidney and urinary bladder of rats one week after infection with equal numbers of isogenic *E. coli* strains expressing either common type I-fimbriae (Pil$^+$) or S-fimbriae (Sfa$^+$).

E. coli strain	Type of fimbriae[a]	Antibiotic marker[b]	Bacterial counts[c] bladder	kidney
536/21 pANN801-4	Sfa$^+$	Tc^r	2.880 ± 20	4.470 ± 10
536/21 pGB30	common type I (Pil$^+$)	Ap^r	72.400 ± 37	740 ± 27

[a] Sfa, S fimbrial adhesion; common type I fimbriae mediating mannose sensitive hemagglutination and pili formation (Pil$^+$).
[b] Tc^r, tetracyclin resistance; Ap^r, ampicillin resistance, each coded by the recombinant plasmid.
[c] Colony forming units/g tissue, mean ± standard deviation.

1). On the basis of these experiments Sfa$^+$ *E. coli* seem able to infect both bladder and kidney but to a different degree.

The fact that S-fimbriae contribute to virulence in a rat UTI model has already been demonstrated on the basis of infections with a Sfa$^+$ strain in comparison to a non-fimbriated strain.[9,10] It has recently also been shown[11] that S-fimbriae are important in sepsis of mice caused by 018:K1 strains. This *in vivo* system is, however, quite different from the UTI model used and it is possible that S-fimbriae have different roles in these diseases. However, although S-fimbriae promote virulence in the rat UTI model used here, S-fimbriated strains are not common in UTI of man, although these fimbriae can effectively bind to human renal tissue cells.[12] This could be due to the presence of oligosaccharides in the human urine which may inhibit S-fimbrial binding to uroepithelial cells[13] (Korhonen, personal communication). The P-fimbriae, however, which

are able to recognize the 2-*D*-Gal-(1-4)-*β*-*D*-Gal part of globosides as a receptor in mouse and man[14] did not act as a virulence factor in this rat pyelonephritis model.[10] P-specific di-Gal-globosides are in fact present on rat cells isolated from the small intestine[15] but missing on rat uroepithelial cells.[16] Thus, the urinary tract cells remain unrecognized by P-fimbriae.

It is further demonstrated here that *E. coli* strains which produce common type I fimbriae (Pil) preferably colonize the mucosa of the rat urinary bladder. Similar observations have also been made in a mouse short term infection model.[14] It is known that type I-fimbriae bind to uroepithelial cells of rats[14] and to the Tamm-Horsfall protein.[17] Since the Tamm-Horsfall protein is excreted by tubular cells into the urine, binding to this protein might be of a disadvantage for colonization of the urine tract. This obviously is outweighed by adhesion to the uroepithelial cells of the inner surface of the bladder. We therefore assume that the Pil$^+$ fimbrial adhesion is a virulence factor in lower urinary tract infections.

Material and methods

The *E. coli* strain 536/21 used here is a non-hemolytic (Hly$^-$), non-fimbriated 06:K15 derivative of the uropathogenic isolate 536.[18] The recombinant plasmids pANN801-4 (Tc^r) and pGB30 (Ap^r)[8] (Hacker *et al.*, in preparation), the former coding for the S-fimbrial adhesion (Sfa) and the latter for common type I-fimbriae (Pil$^+$), were introduced into 536/21 by transformation. The recombinant strains were characterized by genetic and bacteriological standard techniques.[4]

Female Wistaɾ rats (Han WIST) which were 6 to 8 weeks old, were used in these experiments. The rats were fed with Altromin and tap water was supplied *ad libitum*. UTI was induced by transurethral injection of a mixture of both strains (1.5 ml per rat, 10^7 cfu/ml) into the bladder of the rats. One week after infection the animals were sacrificed, the kidney and bladder removed. The bladder was opened and thoroughly washed in sterile saline in order to remove the bacteria that were not firmly attached. Bacterial cell counts were made on McConkey agar containing the appropriate antibiotic (20 mg/l of tetracycline or ampicillin) allowing the growth of the Sfa$^+$ or the common type I-positive strain. The presence of the different fimbriae were checked by hemagglutination and agglutination with specific antisera.[8] The number of bacterial counts per g of tissue was determined and log transformed. The mean values and standard deviation were calculated from the log numbers for each group. In addition the difference between the counts of the Sfa$^+$ and Pil$^+$ strain was calculated for each organ.

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