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THE IMPORTANCE OF *ESCHERICHIA COLI* FIMBRIAE IN URINARY TRACT INFECTION

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ABSTRACT

Urinary tract infection (UTI) is a major bacterial infectious disease among women. Uropathogenic *Escherichia coli* is the most dominant causative agent. Clinical observations indicate that repeated cystitis induces a protective immune response and secretory IgA has been suggested as one of the candidates involved in the defence mechanisms against bladder infections.

The aims of the present study were to study the importance of *E. coli* fimbriae in the pathogenesis of urinary tract infections and especially the role of the P-fimbria PapG class II tip adhesin in the adherence of *E. coli* to cultured human urogenital cells, the importance and protective effect of the PapG class II adhesin in establishing acute cystitis in an experimental model, and antibody response following experimental bladder infections. In addition we studied the induction of protective immunity via immunization with a fragment of the Type 1-fimbria adhesive FimC/H protein.

We have demonstrated P-fimbriated specific adhesion *in vitro* to cultured human urogenital cells in both light microscopy and flow cytometry analyses. The PapG class II positive *DS17* and *JR1* adhered avidly, while the two mutants lacking PapG class II did not. We were able to inhibit adhesion with a soluble digalactoside-containing receptor analogue, indicating the specificity of the PapG-mediated adhesion.

In an experimental cystitis model in primates, both the PapG class II positive *E. coli* strain *DS17* and its two PapG class II negative mutants were able to induce bladder infections. *DS17*, but not the two mutants, gave rise to protection against subsequent bladder inoculation with both *DS17* and the non-adhesive mutants. The acquired protection was correlated to a local production of secretory IgA in urine. In addition, a rise in IgA and IgG was observed in serum. Since the P-fimbriae are expressed in less than 50% of *E. coli* isolates causing cystitis, but the Type 1-fimbriae in a vast majority of these strains, we immunized monkeys with a FimH/C fragment derived from the Type 1-fimbria components. Three of the four immunized monkeys were protected against bladder infection when challenged with the Type 1-fimbriated strain *NU14*. One monkey developed a partial infection. None of four control monkeys were protected when challenged (one partial infection). The protection was correlated to levels of IgG against FimH in serum as well as in vaginal secretions. Furthermore, antibodies in serum and urine were able to inhibit adhesion of the strain *NU14* to cultured bladder cells.

Our conclusions are that *E. coli's* virulence factors P- and Type 1-fimbriae are important in the pathogenesis of urinary tract infection. The PapG class II tip adhesin of P-fimbriated *E. coli* mediates specific adhesion to urogenital cells. A bladder infection with a PapG class II positive *E. coli* strain induces protection against subsequent challenge with homologous strain, while PapG class II negative mutants do not. The protective immune response following experimental bladder infections with a PapG class II positive strain correlates to levels of secretory IgA in urine. Immunization with a fragment of the FimC/H protein derived from *E. coli* Type 1-fimbriae mediates protection against cystitis caused by *E. coli* in a primate model.

Our results contribute to the understanding of the pathogenesis of lower urinary tract infections, and their induction of an immune response. This knowledge should be useful in future vaccine strategies.

PUBLICATIONS

This thesis is based on the following original papers that will be referred to in the text by their Roman numerals.

- I Söderhäll M, Bergerheim USR, Jacobson SH, Lundahl J, Möllby R, Normark S, Winberg J. Molecular evidence for PapG specific adhesion of *Escherichia coli* to human proximal tubular cells. *J Urol.* 157(1):346-350, 1997.
- II Söderhäll M, Normark S, Ishikawa K, Karlsson K, Teneberg S, Winberg J, Möllby R. Induction of protective immunity after *Escherichia coli* bladder infection in primates. Dependence of the globoside-specific p-fimbrial tip adhesin and its cognate receptor. *J Clin Invest.* 100(2):364-372, 1997.
- III Söderhäll M, Colque-Navarro P, Jacobson SH, Möllby R. Antibody response elicited by *Escherichia coli* bladder infection in primates. Manuscript.
- IV Langermann S, Möllby R, Burlein JE, Palaszynski SR, Auguste CG, DeFusco A, Strouse R, Schenerman MA, Hultgren SJ, Pinkner JS, Winberg J, Guldevall L, Söderhäll M, Ishikawa K, Normark S, Koenig S. Vaccination with FimH adhesin protects cynomolgus monkeys from colonization and infection by uropathogenic *Escherichia coli*. *J Infect Dis.* 181(2):774-8, 2000.

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ABBREVIATIONS

ABU	Asymptomatic bacteriuria
bp	base pair
CFU	Colony forming unit
<i>E. coli</i>	<i>Escherichia coli</i>
ELISA	Enzyme-Linked ImmunoSorbent Assay
FITC	Fluorescein isothiocyanate
HA	Haemagglutination
IL	Interleukin
LPS	Lipopolysaccharide
MFI	Mean fluorescence intensity
MRHA	Mannose-resistant haemagglutination
MSHA	Mannose-sensitive haemagglutination
PBS	Phosphate buffered saline
PMT	Photo Multiplier Tube
SC	Secretory component
sIgA	Secretory IgA
TGF- β	Transforming growth factor- β
TNF	Tumor necrosis factor
UTI	Urinary tract infection

INTRODUCTION

URINARY TRACT INFECTION

Urinary tract infection (UTI) is one of the most common bacterial infections among humans, especially in women. UTIs cause severe morbidity, suffering and high costs, both for the individual patient and for society. A growing bacterial resistance against several antibiotics used in the treatment of UTIs has been described (1-4).

The incidence of symptomatic UTI is 0.9-1.4% per year in girls under the age of six years (5) and 0.5-0.7% in adult females who are at highest risk. About 40% of adult women in the United States experience at least one UTI during their lifespan (6) resulting in about 7 million outpatient visits per year (7). 20-50% of adult women have recurrent episodes of UTI and 20% of women who have one UTI will have more than three recurrences within a year (8, 9).

The incidence of symptomatic UTI in men is very low, even though an increase to a prevalence of 6 -15% of bacteriuria in men over 70 years old is seen due to prostatic diseases (10).

UTI AND SOCIO-ECONOMICAL ASPECTS

It has been reported that each period of UTI in young women in the United States is associated with 6.1 days of symptoms, 2.4 days of restricted activity, 1.2 days in which they are not able to attend to work or classes, and 0.4 days in bed (11). The estimated cost for the care of each episode of UTI in adults is 40-80 USD without taking the loss of working days into account (12). The estimated annual health care cost was > 1 billion USD early in the last decade (7) and the estimated annual cost of UTI cases with prescriptions was 1.6 billion USD in 1995 (13). UTI accounts for 15% of all antibiotic prescriptions in outpatient clinics (14). An incidence rate of 2.39 cases of nosocomial UTIs per 100 acute hospital admissions has been documented (15, 16). It has been estimated that a nosocomial UTI prolongs the length of hospital stay by 2 days on average (0.6 – 5 days) (17). Furthermore, in a prospective study, an increased relative risk of death was observed among these patients (18).

PATHOGENESIS

The urinary tract is normally a sterile milieu. The source of uropathogenic bacteria is often the faecal flora which spread via the perineal, vaginal and periurethral areas to the lower urinary tract where they may establish a colonization (19).

Both virulence factors in the bacteria and individual host defence mechanisms in man will decide the outcome of the infection (20). When only the lower urinary tract becomes affected with symptoms such as dysuria and frequency, the infection is defined as an acute cystitis. However, among these patients with symptoms of an acute cystitis, it has been reported that 15-25% also have evidence of occult infection in the upper urinary tract (21). If the infection ascends and also affects the upper urinary tract with symptoms such as flank pain, fever, and malaise, the infection is defined as an acute pyelonephritis, a more severe and, in some cases, a life-threatening disease. The scenario also includes an asymptomatic involvement of the lower urinary tract, asymptomatic bacteriuria (ABU) that can persist for long periods of time.

Complicated UTIs are, by definition, infections in which an underlying structural or neurological lesion exists. The term chronic pyelonephritis is still, somewhat misleading, used as a diagnosis for non-infectious parenchymal scars in one or both kidneys, correlated to upper UTIs in childhood and carrying a risk to develop kidney failure later in the life.

ETIOLOGY

The most common cause of UTI is Gram-negative bacteria that belong to the family *Enterobacteriaceae*. Members of this family include *Escherichia coli*, *Klebsiella*, *Enterobacter*, and *Proteus*. Also the Gram-positive *Staphylococcus saprophyticus* plays a role in the bacterial panorama, especially among young women. *E. coli* dominates as causative agent in all patient groups. In uncomplicated UTIs, 80-90% are caused by *E. coli*, (10, 22) while in younger women 5-20% are caused by *Staphylococcus saprophyticus* (seasonal dependence) (23). In complicated UTIs, *E. coli* is less prominent but still the major causative agent (24).

UROPATHOGENIC *E. coli* AND VIRULENCE FACTORS

Uropathogenic *E. coli* clones are selected subsets of the faecal flora that possess different virulence attributes that enable them to colonize the urinary tract. It is possible to classify uropathogenic *E. coli* with help of cellular markers (serotyping). It is well known that certain lipopolysaccharides (LPS), capsular and flagellar antigens (O:K:H) (25, 26) are associated with symptomatic infections such as acute pyelonephritis, and others with ABU. Other virulence factors of importance in the pathogenesis of UTI are adherence structures (P-, Type 1-, S-fimbriae), haemolysin, aerobactin, invasion factors and serum resistance (27). Many virulent genes are clustered on “pathogenicity islands”, which are large chromosomal regions that often are associated with particular tRNA loci (28). The expression of these factors is often co-regulated, and the regulation is sensitive to different environmental signals.

P-FIMBRIAE

P-fimbriae are rod-like rigid heteropolymeric appendages that protrude from the cell-surface of *E. coli* bacteria. The P-fimbriae are encoded by the *pap* chromosomal gene cluster. The major bulk subunit is composed of the PapA protein, which has a shaft-like structure. This is anchored to the cell membrane with PapH (29). The thin flexible tip is composed of four different subunits where PapK connects the adhesive PapE/PapF/PapG to the shaft PapA. The binding adhesin, PapG, is located on the tip of the fibrillum (30, 31). Three classes of PapG adhesins with different variants of receptor specificity and different clinical relevance have been described (Table 1) (32, 33). The different PapG adhesins bind to isoreceptors of glycolipids in the urinary tract which all contain the disaccharide Gal α [1-4]Gal β , but its position in the glycolipid molecule differs. PapG class I has no known clinical relevance in humans. The PapG class II adhesin recognizes globoside and attaches to uroepithelial cells and erythrocytes from all P blood group positive individuals. The class III adhesin binds to the Forssman antigen and to globoA. It has been demonstrated that UTI caused by P-fimbriated *E. coli* expressing the PapG class III adhesin is more common in blood group A₁P₁ secretor positive humans. These individuals have been shown to carry globoA on their uroepithelial cells (33).

Thus, the P-fimbriae mediate mannose-resistant haemagglutination (MRHA) of erythrocytes from individuals with the common bloodgroup P₁, but fail to agglutinate erythrocytes of the more uncommon blood group p (34, 35).

The expression of P-fimbriae on the bacterial surface is controlled by phase variation. The phase variation *in vivo* is still unclear (36) but, *in vitro*, different conditions such as changes in glucose concentration, pH and temperature may switch the P-fimbriae on or off (37, 38). In clinical isolates, the *papG* gene is identified in 80-90% of the strains in acute pyelonephritis, 50-60% in cystitis and ABU and 10-20% in the normal faecal flora (39).

P-fimbriae mediated adherence to the oligosaccharide receptors induces epithelial cell activation and production of cytokines such as IL-6 and IL-8 (40).

Table 1. Characteristics of the three PapG adhesin variants (41)

PapG variant class	Clinical relevance in humans	Glycolipid Receptor preferences	Haem-Agglutination of erythrocytes
I	Not known	Ceramide trihexoside	Human, Rabbit
II	Dominates in acute pyelonephritis	Globoside	Human
III	Occurs in acute cystitis	Forssman antigen, globoA	Sheep, Dog

TYPE 1-FIMBRIAE

Type 1-fimbriae are widely spread among different *E. coli* strains causing UTI (42, 43). They mediate adhesion to secreted and cell-bound mannosylated glycoproteins and exhibit mannose sensitive haemagglutination (MSHA) of guinea pig erythrocytes (44), (45). Addition of D-mannose inhibits this haemagglutination. The Type 1-fimbriae are encoded by the chromosomal *fim* gene cluster. In similar manner to the P-fimbriae, the major subunit of the Type 1-fimbriae consists of FimA (46) and it is arranged in a tight right-handed helical rod (47). There are at least three minor pilus proteins that are organized into structures seen on the end of the fimbria, FimF, FimG and the specific binding adhesin FimH (48, 49). The assembly of the Type 1-fimbriae depends on two chaperone proteins that do not form a part in the final structure: FimC and FimD (50, 51).

Phase variation controls the expression of Type 1-fimbriae by site-specific recombination. A 314-bp phase-variable invertible element, containing the promoter, in the gene cluster controls the transcription of the fimbrial genes *fimACDFGH*. The promoter drives the expression of the Type 1-fimbriae when the switch is in the ON orientation but not when it is in the OFF orientation (52, 53). It has recently been shown in mice that during acute cystitis, a significantly larger percentage of the isolates kept their invertible element in the ON phase compared to isolates during acute pyelonephritis (54).

Receptor-epitopes for Type 1-fimbriae binding FimH adhesins are present on a variety of cells, such as buccal, tubular, urethral, and vaginal cells from different species (44, 55). The FimH protein also adheres to the Tamm Horsfall protein present on the mucosa in the human urinary tract. Wu *et al.* have shown that Type 1-fimbriae *in vitro* bind to mucin and uroplakin, which the latter is crystalloid structures deposited on the uroepithelial cells (56). It has been suggested that Type 1-fimbriae may be more important in bladder colonization than P-fimbriae. Schilling and colleagues have previously shown that the FimH adhesin mediates invasion of the superficial bladder epithelial cells and, via LPS, induces an IL-6 response, which may be important in the host response to acute cystitis (57).

HOST FACTORS IN URINARY TRACT INFECTIONS

Host factors involved in bacterial clearance can be divided into: 1) natural host resistance factors existing prior to bacterial challenge, and 2) acquired host resistance factors that are activated in response to bacterial infections.

The normal periurethral bacterial microflora is a part of the resistance against colonization with pathogenic bacteria. It makes up the first line of defence against ascending UTIs. It has been shown that females who are prone to UTI harbour larger numbers of *Enterobacteriaceae* even during infection-free periods, as compared to healthy subjects (58, 59)

In healthy individuals, the urinary flow and the regular emptying of the bladder are important defence mechanisms. The mucous layer covering the bladder epithelium and slime soluble in the urine, prevent bacterial attachment and probably enhance the effectiveness of bladder washout (60, 61).

The uroepithelium normally has a low regeneration turnover. However, in UTI an increased rate of exfoliation is seen, which removes adherent bacteria (62, 63).

Specific immunity

The role of specific immunity in UTI remains unclear. Elevated titers of serum and urinary antibodies have been detected after UTIs. These antibodies consist mainly of IgM and IgG in serum and secretory IgA (sIgA) in urine (64, 65). Both induced antibodies directed against LPS, e.g. the O antigen, as well as those directed to adhesive structures such as the P- and Type 1-fimbriae have been detected (66-69). Type specific protection has been demonstrated in experimental animals immunized with O, K antigens or P-fimbriae (70, 71).

The main portion of urinary sIgA originates from the bladder and the urethra where it is secreted by the urothelium (72). It has been proposed, but little has been shown, that urinary sIgA may play a major role in the local defence mechanisms of the human urinary tract (73). It has further been suggested that urinary sIgA inhibits bacterial colonization by lowering the bacterial adherence to the mucosa (67, 74). Svanborg-Eden *et al.* showed that adhesion of *E. coli* to human urinary tract epithelial cells was inhibited by sIgA fractions of urine from patients with acute pyelonephritis (68). Hopkins *et al.* observed that the cystitis resolution, in primates, is correlated to levels of urinary IgA and IgG antibodies (75).

The secretory IgA system is common to all mucosal surfaces. sIgA is the mutual product of plasma cells and mucosal epithelial cells. The B cells which are first exposed to antigens in mesenteric lymph nodes and at mucosal surfaces migrate through the lymphatic vessels into the systemic circulation prior to differentiation. Thereafter the B cells “home” to the lamina propria beneath the mucosal surface and differentiate into IgA-secreting cells. As a late event in synthesis, the J-chain, a 15-kDa protein, initiates the polymerization of two sIgA monomers and becomes incorporated into the molecule (76, 77). The secreted dimeric IgA is then transported across the epithelial cells by endocytosis initiated by binding to a poly-Ig receptor. At the apical surface of the epithelial cells, the poly-Ig receptor is cleaved enzymatically and becomes the secretory component (SC), a 80-kDa transmembrane glycoprotein. The dimeric IgA is then released into the mucous secretion bound to SC (78-80).

Innate immunity

Mucosal inflammation is an important factor for clearance of the bacteria from the urinary tract and linkage has been demonstrated between pyuria and eradication of bacteria (81).

Cytokines are small proteins that mediate signals between cells and play an important role in the regulation of the host defence against bacterial infections (82). Cytokines such as tumour necrosis factor (TNF), Interleukin (IL)-1, IL-6 and IL-8 are produced from local epithelial cells, macrophages, leukocytes and endothelial cells. IL-8 is a chemokine with chemotactic properties for neutrophils.

Earlier studies have demonstrated that UTI is accompanied by a cytokine response both in mice with experimental UTI as well as in patients who had been colonized with *E. coli* in the urinary tract (83-85). IL-6 has been found in the urine of children with febrile UTI and the highest levels in urine and serum were detected in children with reflux and renal scarring (86). In adults, IL-6 was detected in the urine of patients with acute pyelonephritis and ABU (85). Furthermore, in women and children suffering from acute pyelonephritis, IL-6 and IL-8 have been detected in urine (87, 88)

Patients deliberately colonized with *E. coli* in the urinary tract secreted IL-8 into the urine within hours of colonization while serum IL-8 was not detected (84). Urinary IL-8 has also been detected in patients with pyuria due to acute pyelonephritis or ABU (89). In mice, mRNA for proinflammatory and regulatory cytokines (IL-1, IL-6, TNF- α , IL-4, IL-10, transforming growth factor β (TGF- β)) has been demonstrated following experimental acute pyelonephritis (90), as well as secretion of urinary IL-1 α and IL-6 (91).

Epithelial cell-lines and primary cell cultures from the urinary tract produced cytokines when exposed to uropathogenic *E. coli* and production of IL-1 α , IL-1 β , IL-6 and IL-8 was detected (84, 88, 92, 93).

Thus, both locally secreted and systemically generated cytokines seem to play an important role in the host defence against UTI.

Vaccines against UTI

Vaccination trials using systemically, perorally or vaginally applied antigens from uropathogenic *E. coli* have indicated possible protection against recurrent cystitis (94-97). Roberts and coworkers have shown that immunization with purified P-fimbriae gave rise to protection against acute pyelonephritis in a primate model (71). It has been proposed that the FimH adhesin may be used as a common antigen in the development of vaccine against UTI. *In vivo* studies in mice have demonstrated that immunization with FimH results in a significant protection against challenge with a Type 1-fimbriated *E. coli* strain (98). Induced anti-FimH antibodies blocked *in vitro* attachment of *E. coli* to bladder cells and the concomitant inflammatory response (98).

THE PRESENT STUDY

AIMS

To study the importance of *Escherichia coli* fimbriae in the pathogenesis of urinary tract infections. The specific objectives of the investigations were:

- to study the specificity of PapG class II adhesin in the adherence of *E. coli* to cultured human proximal tubular cells
- to study the importance of the PapG class II adhesin in establishing acute cystitis in an experimental primate model and its protective effect against subsequent bladder infection.
- to further study the antibody response following experimental bladder infections in primates and specifically the influence of the PapG class II adhesin.
- to compare the systemic and local immune response elicited by induced cystitis and correlate that to the protective effect.
- to study the induction of protective immunity against *E. coli* bladder infection via immunization with fragments of the FimC/H protein.

MATERIAL AND METHODS

Cultured human urogenital cells

Human renal cortical tissue was obtained from nine patients suffering from renal cell carcinoma and undergoing elective total nephrectomy. The extreme outer cortex from unaffected tissue was excised, decapsulated and minced. The tissue fragments were incubated overnight in culture medium with collagenase VIII (1 mg/ml) at 37° C in 5% CO₂-95% air. The culture medium consists of RPMI 1640 supplemented with 10% heat inactivated fetal calf serum, 2 mM L-Glutamine, 10 mM HEPES, benzyl-penicillin (100 U/ml), and streptomycin (100 µg/ml). Half of the culture medium was changed regularly and the cell culture reached confluence in 10-14 days. The cell cultures were characterized as proximal tubular cells by their typical morphology appearance in light- and electron microscopy. They were then stained histochemically and they exhibited positive staining for cytokeratine and negative staining for the von Willebrand factor, indicating an epithelial origin.

Cultured urethral cells were recovered from one of the nine patients. The luminal surface of the ureter was gently scraped with a scalpel and the cells were cultured in the same manner as described above. In addition, renal cancer cell cultures were obtained and used from two patients in parallel with two established bladder cell lines, T24 and J82 (ATCC, Manassas, VA, USA).

***Escherichia coli* strains**

DS17 is a strain of *E. coli* isolated during an epidemic outbreak of pyelonephritis in a neonatal ward (99). The strain is of serotype O6:K5:H-. *DS17* carries a *pap* gene cluster encoding P-fimbriae with a class II PapG-adhesin. When the strain is cultivated on colonizing factor agar (CF-agar), the expression of P-fimbriae is optimised and the expression of Type 1-fimbriae depressed. The strain also has the capacity to express Type 1-fimbriae when cultivated in static broth. It lacks S-fimbriae and Afa-1 adhesin and, it produces haemolysin and aerobactin. The strain is sensitive to ciprofloxacin, but is resistant to ampicillin and trimethoprim sulphonamide.

DS17-1 is mutant derivative made from *DS17*. It was created through a substitution of the *papG* class II allele, with a gene encoding for PapG class III, which binds to the Forssman antigen (100, 101). This class-switch mutant agglutinates sheep erythrocytes but not human erythrocytes (101). *DS17-1* is functionally identical to the wild type *DS17* in all

respects, except for the binding capacity mediated by PapG.

DS17-8 is a second mutant derivative made from *DS17*. It was created through the introduction of a 1-base pair deletion early in the *papG* gene (100, 101). It expresses P-fimbriae, but not the specific binding PapG adhesin, and it is therefore unable to mediate any Gal α (1-4)Gal-specific attachment, and it is accordingly negative for haem-agglutination. *DS17-8* is also functionally identical to the wild type *DS17* in all respects, except that it lacks the binding capacity mediated by PapG.

JR1 is a wild type *E. coli* strain. The serotype is O4, K:NT,H4. It expresses P-fimbriae with a PapG class II adhesin and Type 1-fimbriae, and it produces haemolysin (75).

NU14 is a wild type *E. coli* strain isolated from a patient with recurrent cystitis (102). It expresses excessively Type 1-fimbriae (both cultured on agar and in static broth) but not P-fimbriae and it agglutinates both guinea pig erythrocytes and yeast cells.

NU14-1 is a mutant derivative made from *NU14*. A chloramphenicol cassette was recombined into the *fimH* gene in the chromosome of NU14, creating a FimH tip adhesin negative mutant. *NU14-1* fails to agglutinate both guinea pig erythrocytes and yeast cells (98).

Table 2. Fimbriae expression capacity in the different bacterial strains

Bacterial strain	P-fimbriae		Type 1-fimbriae
	PapG class II	PapG class III	FimH
<i>DS17</i>	+	-	+*
<i>DS17-1</i>	-	+	+*
<i>DS17-8</i>	-	-	+*
<i>JR1</i>	+	-	+*
<i>NU14</i>	-	-	+
<i>NU14-1</i>	-	-	-

* Must be cultured in static broth to optimise the expression of Type 1-fimbriae.

Adhesion studies on cultured cells

The cultured human proximal tubular cells were seeded on two-wells chamber slides (Nunc, Roskilde, Denmark) in a concentration of 5×10^5 - 10^6 cells/ml and cultured until subconfluency, washed 2 x 5 min. with phosphate buffered saline (PBS), incubated with bacterial suspension under slow agitation for 60 min. and then washed 5 x 5 min. with PBS. The glass slides were fixed in methanol, May-Grünwald-stained, mounted, and adhesion was evaluated using a light microscope, and assessed as positive or negative. Positive evaluation was characterized by abundant adhesion of bacteria, while in negative experiments no bacteria had bound to the cells. Also bacterial adhesion to cultured urethral cells, renal carcinoma cells, and established bladder cell lines were performed as described above.

Inhibition of bacterial adhesion was examined using a soluble digalactoside-containing cell membrane receptor analogue (ACA-118, Astra Sweden) at a concentration of 4 mg/ml (5.6 mM).

In addition, bacterial adhesion was studied in a more sensitive assay by flow cytometry. Cultured renal cells in monolayer were trypsinated, washed once in PBS, and resuspended in 1 ml of the same buffer to a final concentration of 1×10^6 cells/ml. An equal volume of FITC-labelled bacteria in suspension (5×10^8 CFU/ml) was added, slowly agitated for 45 min. at room temperature and then washed 5 x 5 min. with PBS. The cells were analysed in Epics Profile II (Coulter Inc., Hialeha, FL, USA). The renal cells were identified and separated by their different light scattering properties. The flow cytometer gave the mean fluorescence intensity (MFI) units for the selected cell population. The Photo Multiplier Tube (PMT) setting permitted clear-cut distinctions between specific and non-specific binding. Within the selected cell-population, a minimum of 10,000 cells was analysed.

Monkeys

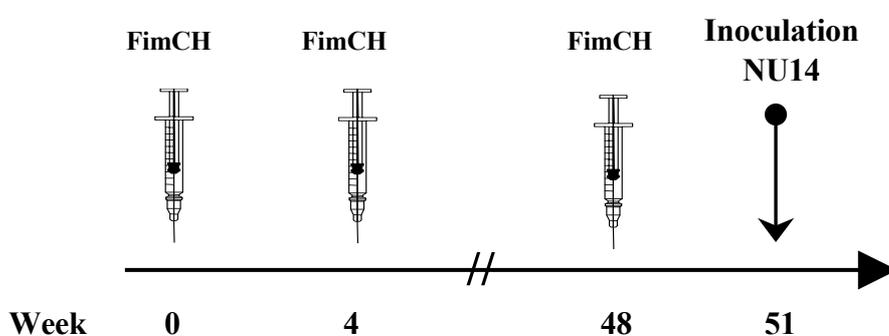
All the monkeys were cynomolgus monkeys (*Macaca fascicularis*, healthy, adult, female). 22 out of the 41 monkeys were inbred and the other 19 outbred, imported from China and the Philippines. The monkey's anatomy, bacterial flora and cellular glycolipid patterns are similar to those in humans. However, the urethra terminates in the vagina and they are not separate orifices as in humans. They monkeys were housed separately with free access to water and food during periods of experiments.

FimC/H immunization

From the *E. coli* strain *NU14*, a complex composed of the periplasmic chaperones FimC (22.8 kDa) and FimH (~29.1 kDa) in a 1:1 equimolar ratio, was expressed in the *E. coli* strain K12. The complex was extracted from the periplasm and purified to >99% purity.

Upon immunization, four monkeys were injected intramuscularly at weeks 0, 4 and 48 with the FimC/H vaccine in adjuvant MF59 (Chiron, Emeryville, CA) and four control monkeys received only the adjuvant in the same manner. The monkeys were challenged with the Type 1-fimbriated strain *NU14* 18 days after the final immunization (u 1).

Figure 1. Schematic schedule of the vaccination study.



Induction of bladder infection

The monkeys were housed separately. Water was withheld 8 hours prior to the experiments. All experiments were done under ketamine and metazolam anesthesia. Bladder infection was induced through inoculation of bacterial suspension (1×10^7 CFU/ml) via an urethral catheter. Blood samples and suprapubic bladder urine aspirations were performed twice a week until two negative urine cultures were obtained. The urine and serum samples were immediately frozen at -20°C and stored until the time for analysis. In the protection studies, after two negative urine cultures, a subsequent bacterial inoculation was given as described above.

Antibody detection

Levels of IgA, sIgA and IgG in urine and serum were analysed in microtiter plates coated with LPS derived from *DS17* (10 µg/ml, incubated overnight at 22°C.) Serum was diluted 1/400 in PBS-T and urine was tested undiluted. All samples and controls were incubated during one hour at 37°C. Three controls consist of urine / serum with known absorbance values, two positive (high and medium), and one negative and one control with only PBS-T. Conjugates in the respective ELISAs were diluted in PBS-T and incubated for two hours at 37°C.

IgA was analysed with a conjugate (goat anti-monkey IgA Fc-specific; Nordic Immunology, Tilburg, The Netherlands) labelled with horseradish peroxidase, and diluted 1:5000 for serum analysis and 1:1500 for urine analysis. The reaction was developed with ABTS (2', 2'-azino-bis (3ethylbenzthiazoline-6-sulphonic acid) diamonium) (0.22 mg/ml) in citric acid.

Secretory component (SC) in urine was analysed with a conjugate (goat anti-monkey secretory component-specific, both Nordic Immunology, Tilburg, The Netherlands) labelled with horseradish peroxidase, and diluted 1:1500. The reaction was developed with ABTS (0.22 mg/ml) in citric acid (0.05 M, pH 4.0).

IgG was analysed with a conjugate (goat anti-human IgG γ -specific; Sigma Chemical Co., St. Louis, MO, USA) labelled with alkaline phosphatase, and diluted 1:2000. The reaction was developed with para-nitro-phenylphosphatase (1 mg/ml; Sigma) solubilized in diethanolamine (1 M, pH 9.8).

The reactions were measured at 405 nm in microtiter plates. The final reading was performed when the controls achieved the desired absorbance values in the respective ELISA. The data was transmitted on line from the reader to a computer. Mean values of duplicates were calculated with deduction of the blank. The blank was the first uncoated column containing only developing substrate buffer. An immune response in urine or in serum was defined as positive when the peak absorbance value was at least twice the initial base level. All absorbance values < 0.200 were considered as negative.

Vaginal colonization

Bacterial suspension (10^9 CFU/ml, 3ml) was flushed over the monkey vaginal mucosa via a catheter. To obtain vaginal specimens, a sterile cotton swab was rotated against the vaginal wall. The sample was suspended in 1 ml of PBS, vortexed and 0.1 ml of the suspension was cultured on a Cled agar plate. A successful vaginal colonization was defined as persistence of $>10^4$ CFU/ml vaginal fluid for at least 6 subsequent days.

Chromotogram binding assay

Glycosphingolipids, extracted from a cynomolgus monkey, were separated on aluminium-backed silica gel 60 High Performance Thin-Layer Chromatography plates (Merck, Darmstadt, Germany) with chloroform/methanol/water 60:35:8 (by vol.) as the solvent system. The dried chromatograms were treated with 0.5% polyisobutylmethacrylat (Röhm, GmbH, Germany) in diethyl ether (wt/vol), and thereafter soaked in PBS with 2% bovine serum albumin and 0.1% NaN_3 . The chromatograms were then incubated with S^{35} -labelled *DS17* or *DS17-8* for two hours, followed by washing with PBS. Autoradiographies were performed using x-ray film.

RESULTS

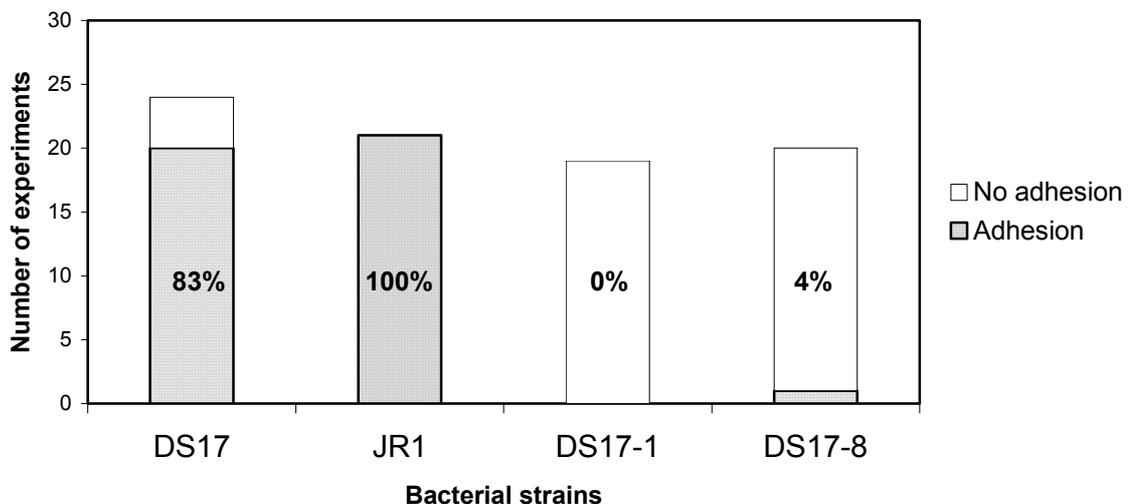
Bacterial adhesion to cultured human urogenital cells (I)

Light microscopy

Experiments were performed on urogenital cells from nine patients. The wild type strain *DS17* adhered in 20 of 24 experiments and the wild type strain *JR1* in all of 21 experiments (Fig 2). The PapG class III positive mutant *DS17-1* adhered in none of the 19 experiments, while the PapG negative mutant *DS17-8* adhered in one of the 23 experiments.

A similar pattern was observed in adhesion of bacteria to cultured renal cancer (n= 2), cultured urethral cells (n= 1) and established bladder cell lines (n=2) (data not shown).

Figure 2. Adhesion of uropathogenic PapG class II positive *E. coli* and mutants lacking the papG class II adhesin to human proximal tubular cells.



Flow cytometry

Adhesion of FITC-labelled bacteria to human proximal tubular cells in suspension from six patients was studied. After incubation with *DS17* and *JR1* a much higher proportion of high fluorescence was quantified compared to adhesion with the PapG class II negative mutants. This indicates significantly higher binding capacity of PapG class II positive strains compared to the mutants lacking this tip protein structure.

Inhibition of bacterial adherence

The digalactoside-containing soluble cell membrane receptor ACA-118 totally inhibited bacterial adherence of *DS17* and *JR1* to proximal tubular cells at a concentration of 5.6 mM.

Together, these findings indicate that the PagG class II tip adhesin is essential for bacterial adherence to human urogenital cells.

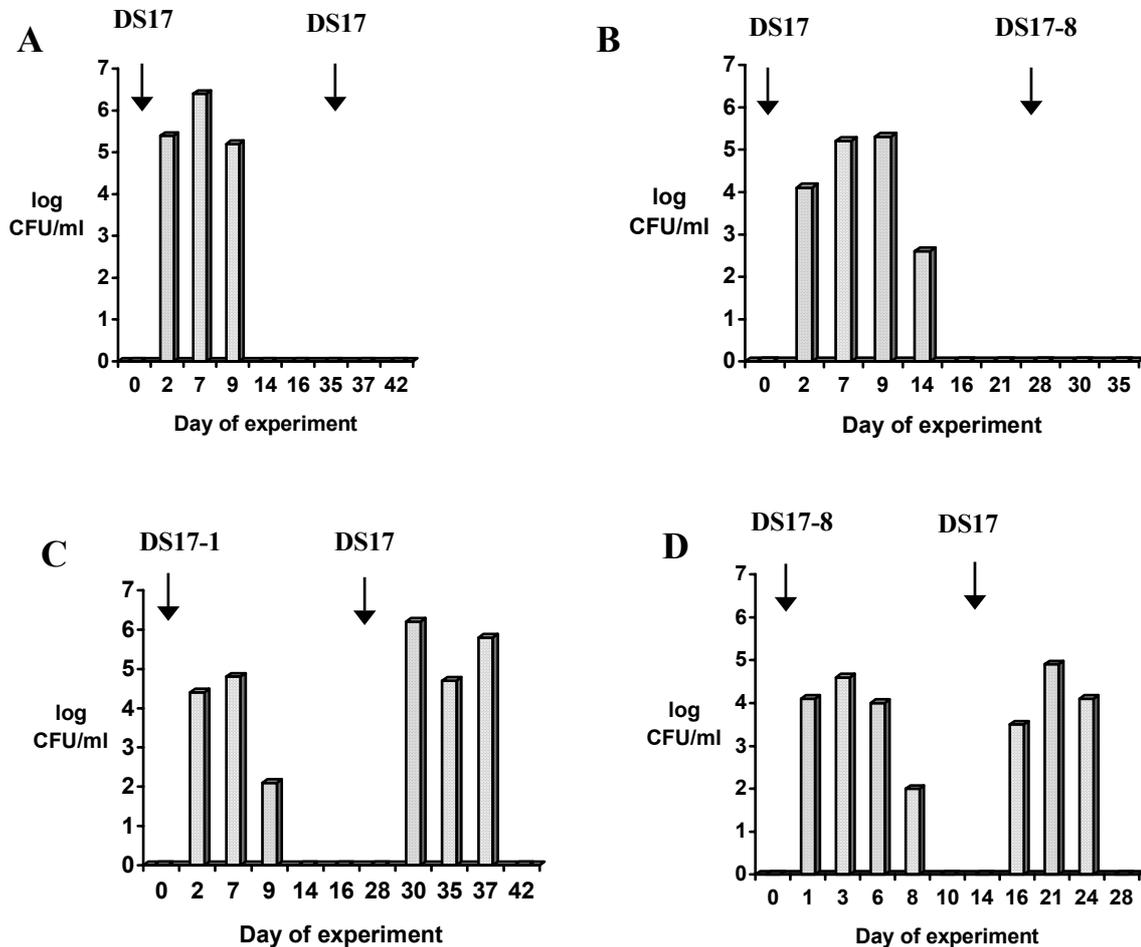
***DS17* protects against subsequent bladder infection (II)**

Both the wild type strain *DS17* and its two isogenic mutants induced bladder infection at primary inoculation in 23 out of 24 monkeys. One monkey, primarily inoculated with *DS17-1*, voided directly after the inoculation and did not achieve any infection. Reinoculation with the same strain induced a partial infection. The median duration of infection caused by the three strains was not significantly different.

For comparison of bacterial counts in infections caused by the three different strains, the first three urine cultures were selected. *DS17* gave rise to infections with significantly higher bacterial numbers (median 4.1×10^5 CFU/ml) compared to *DS17-1* (median 2.9×10^4 CFU/ml) and *DS17-8* (median 4.5×10^4 CFU/ml) ($p < 0.05$, Mann-Whitney ranking test). No significant differences were found between the strains in leukocyte esterase tests or polymorph white blood cell counts on day two.

After the first infection, followed by two negative urine cultures, the monkeys were subsequently challenged with the same bacterial strain or another of the strains in the study (Fig 3). 11 of 12 monkeys primarily inoculated with the wild type strain *DS17* were completely ($\text{CFU} < 10^2/\text{ml}$ in all urine cultures) or partially ($\text{CFU} \geq 10^2/\text{ml}$ in one, but not two urine cultures) protected against reinfection with both *DS17* and *DS17-8* (Table 3). Neither of the two PapG class II negative mutants inoculated into 10 monkeys gave rise to protection against *DS17*. A group of monkeys primarily infected with one of the two mutants and reinfected with *DS17* were given another challenge with *DS17*. Nine of these 12 monkeys were completely protected against the second challenge with *DS17*. The remaining three exhibited partial protection.

Figure 3. Examples of the four different sets of protection experiments. A and B were primarily inoculated with the wild type strain *DS17* and at challenge protected against subsequent bladder infection with both itself and the mutant *DS17-8*. C and D were primarily inoculated with the PagG class II negative mutants *DS17-1* and *DS17-8* and at challenge not protected against bladder infection with the wild type strain *DS17*.



Furthermore, in series of repeated inoculations we could demonstrate that previous bladder infections with *DS17* also induced protection against the heterologous PapG class II positive strain *JRI* in 12 (one of these partial) out of 19 experiments (63 %).

Table 3. Protection after primary inoculation at subsequent challenge.

First Inoculation	Subsequent inoculation	Number of monkeys		
		Total	Protection	No protection
<i>DS17</i>	<i>DS17</i>	5	4	1 ^a
<i>DS17</i>	<i>DS17-8</i>	7	7 ^b	0
<i>DS17-1</i>	<i>DS17</i>	5	0	5
<i>DS17-8</i>	<i>DS17</i>	5	0	5
Mutant + <i>DS17</i>	<i>DS17</i>	12	12 ^c	0

^a No protection at second inoculation, but complete protection at third inoculation.

^b Partial protection in two monkeys.

^c Partial protection in three monkeys.

Vaginal colonization did not induce local protection (II)

Herthelius *et al.* have earlier shown that the *DS17* strain is an excellent colonizer of the monkey vagina (103, 104). In the present study, successfully repeated vaginal colonizations were performed with both *DS17* and the two mutants in four monkeys. No local protection was observed. The strains were able to spread to the gut, and it was possible to recover them from faeces during the experiment. Thus, neither *DS17* nor the mutants were able to induce local protection in the vagina or the gut.

Adhesion patterns in primate urogenital tissues (II)

Glycolipid preparations from monkey kidney, ureter, bladder and vagina wall were used in a chromatogram binding assay. *DS17* bound to globoside from kidney and bladder. *DS17-1* bound to globoside and to a compound migrating in the pentaglycosyl ceramide region from the kidney and the bladder. It has previously been shown that the PapG class III adhesin binds to globoside immobilized on chromatograms even if it cannot bind to globoside in natural cell membrane (105). *DS17-8* did not exhibit any binding in the assay.

Thus, glycolipid extractions from the bladder wall of cynomolgus monkey reveal the presence of globoside, the preferred isoreceptor for the PapG class II adhesin.

***DS17* elicits antibody response after bladder infection (III)**

Five additional new monkeys were investigated in the experimental cystitis model. By an indirect ELISA, levels of IgA, sIgA and IgG against LPS derived from *DS17* were detected in 263 urine and 230 serum samples from the five new monkeys and 16 of the monkeys described in paper II.

Clinical data

The five new animals were primarily infected with the PapG negative mutant *DS17-8* and cystitis was established in four out of the five monkeys. The fifth monkey acquired a partial infection and was shortly thereafter successfully reinfected with the same strain.

The median duration of the infection was significantly shorter in primary infections caused by *DS17-8* (9 days) in the new monkeys compared with primary infection caused by the wild type *DS17* (14 days) ($p=0.02$, Mann-Whitney ranking test). There were no differences in duration compared to the other primary infections. Bacterial counts were significant higher in the *DS17* series compared to primary infections with all the isogenic mutants ($p < 0.05$, Mann-Whitney ranking test).

After subsequent inoculation with the wild type *DS17*, cystitis was established in four out of the five monkeys. One monkey developed a partial infection and was successfully reinfected with *DS17*. The PapG negative mutant *DS17-8* did not induce protection against the wild type *DS17* in any of the cases.

Antibody detection

Among the 21 monkeys, after primary infection with *DS17* in 11 monkeys, a positive local and systemic immune response was seen in a majority of monkeys with regard to urinary sIgA, serum IgA and, serum IgG (Fig 4).

In monkeys primarily infected with the PapG class III positive mutant *DS17-1*, none of the five monkeys exhibited any positive immune response with regard to urinary sIgA. Three and all of the five monkeys exhibited a positive immune response in serum IgA (60%) and serum IgG (100%) respectively (Fig 4). After the first infection with the PapG negative mutant *DS17-8*, two of the five new monkeys (40%) presented an unexpected positive immune response in urinary sIgA. However, in both these two monkeys a three-fold rise in absorbance values for sIgA was seen after challenge with *DS17*. In serum, a positive immune response for IgA and IgG was seen in one of the five new monkeys (20%). The positive immune response in urine after primary infections with the mutants appears to show lower magnitude compared to primary infections with *DS17* (Table 4).

Figure 4. Immune response after first inoculation with *DS17*, *DS17-1*, and *DS17-8*.

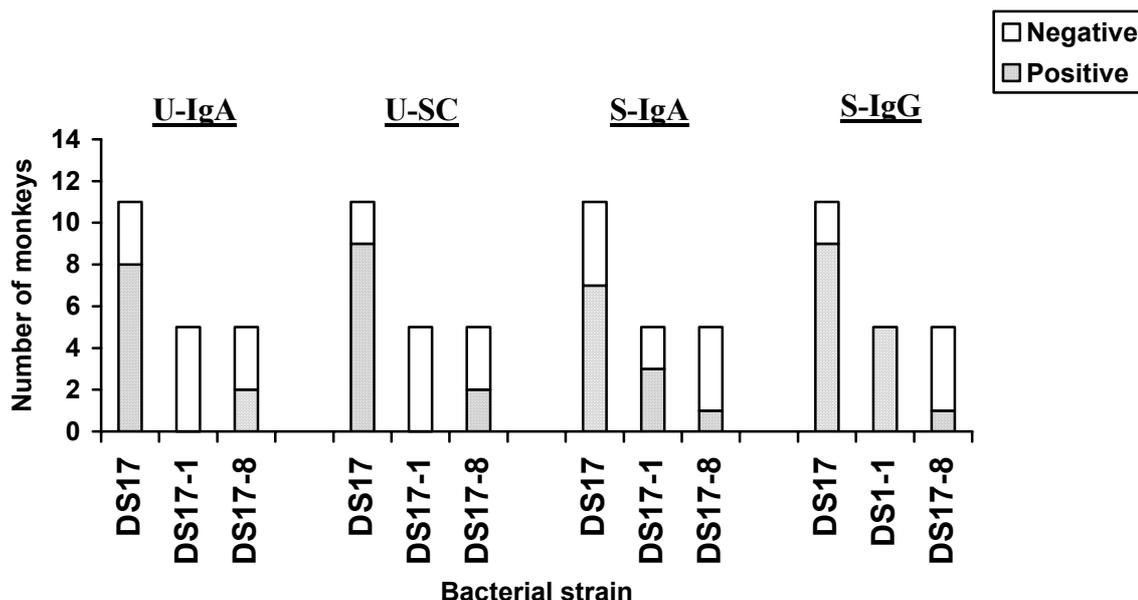


Table 4. Initial and maximum absorbance values in ELISA during first infection. Immune response evaluated as positive is marked in **bolded text**.

Infecting strain	Monkey	Absorbance values							
		U-IgA		U-SC		S-IgA		S-IgG	
		Initial	Max	Initial	Max	Initial	Max	Initial	Max
<u><i>DS17</i></u>	M1	128	168	97	1177	0	296	14	209
„	M2	170	688	117	2062	0	169	13	851
„	M3	57	2175	184	2069	0	864	0	1749
„	M4	331	1182	279	1571	0	703	13	1298
„	M5	106	157	95	168	0	122	47	619
„	M6	127	127	121	199	0	103	60	187
„	M7	110	647	52	425	139	321	726	1230
„	M8	157	1026	33	949	275	1254	221	1373
„	M9	111	224	58	234	0	46	11	321
„	M10	123	883	156	1991	0	357	58	365
„	M11	116	1217	109	831	0	371	11	321
<u><i>DS17-1</i></u>	M12	288	363	426	458	8	201	102	1170
„	M13	0	61	0	16	2	62	56	206
„	M14	81	190	51	175	0	240	65	1089
„	M15	127	219	125	240	0	432	107	1616
„	M16	65	118	130	168	5	40	58	396
<u><i>DS17-8</i></u>	M17	24	264	89	510	18	49	10	52
„	M18	9	313	60	388	2	216	0	372
„	M19	80	198	145	164	18	38	8	51
„	M20	16	32	112	154	76	93	284	515
„	M21	100	104	156	300	16	105	1	196

None of the monkeys primarily infected with the wild type *DS17* showed any further local or systemic immune response in urinary sIgA, serum IgA or serum IgG upon challenge with neither *DS17* nor the PapG negative *DS17-8*. In monkeys primarily infected with any of the two PapG class II negative mutants, eight out of ten monkeys (80%) presented a positive local immune response in urinary sIgA after challenge with the wild type *DS17*. Four out of the ten monkeys exhibited a positive systemic response with respect to serum IgA (40%) and, five with respect to serum IgG (50%).

Detection of urinary IgG was performed in the five new monkeys primarily infected with *DS17-8* and challenged with *DS17*. Urinary IgG was detected with a low absorbance value in one of the five new monkeys after challenge with *DS17*.

Thus, it appears as if bladder infections with the wild type *DS17* enhance a local production of sIgA in the bladder, as well as systemic IgA and IgG.

Direct bacterial agglutination tests

Urine from five monkeys primarily inoculated with *DS17*, and two monkeys primarily inoculated with *DS17-8*, were evaluated. None of the acute samples, prior to infection, were positive for bacterial agglutination. A positive bacterial agglutination was seen in urine from four out of five monkeys after *DS17* infections but in neither of the two monkeys after *DS17-8* infections.

Vaccination with FimC/H adhesin induces protection (IV)

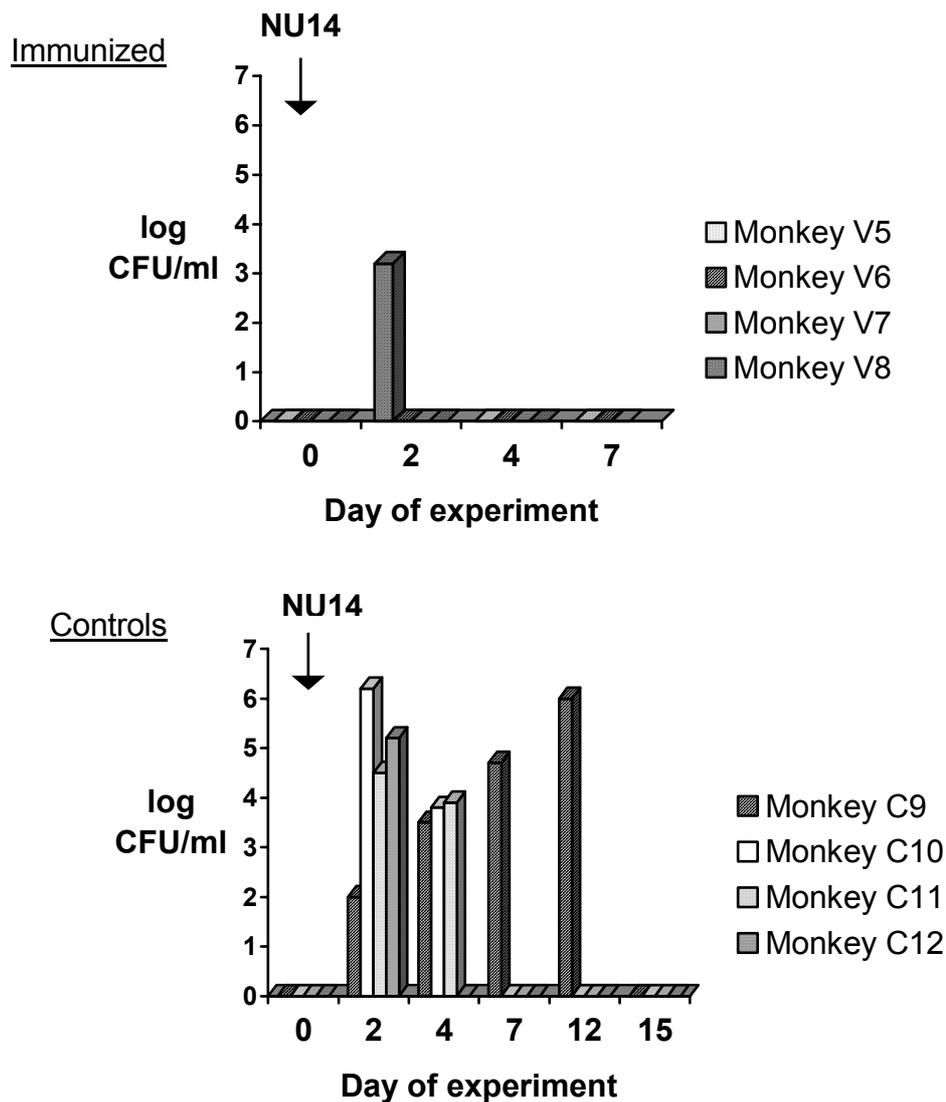
The capacity of the Type 1-fimbriated *E. coli* strain *NUI4* to induce bladder infection was tested successfully in the present study in two monkeys, while an isogenic *fimH* knock-out mutant strain (*NUI4-1*) did not induce bladder infection in another two monkeys.

Four monkeys immunized with FimC/H and four control monkeys were challenged with *NUI4* intravesically. Prior to inoculation, the expression of Type 1-fimbriae in the bacterial suspension was quantified with a FITC-labelled FimH antibody (106). The Type 1-expression was estimated to be 80-90%. Three of the four immunized monkeys were protected against bladder infection with *NUI4*. The fourth monkey developed a partial bladder infection with a single positive urine cultures on day 2. None of the four control monkeys were protected against *NUI4* bladder infections. One of the four monkeys acquired a partial infection with a positive urine culture on day 2 (Fig 5).

An ELISA was performed with the FimH protein fragment T3 as antigen. A systemic IgG response in serum against T3 was detected in all the four immunized monkeys with a final 32- to 256-fold booster response after third immunization. Control monkeys showed no detectable antibodies against T3.

IgG antibodies against FimH were also found in vaginal secretions and increased in parallel with serum levels after booster immunization. Their biological activity was also shown by their ability to block *in vitro* binding of the Type 1-fimbriated *NU14* strain to the established bladder cell line J82. Interestingly, the monkey that was not protected against challenge after immunization, was the only monkey in the vaccinated group that did not have an increase in IgG against FimH in the vaginal secretions.

Figure 5. Bladder infections in the vaccination study after challenge with the Type 1-fimbriated strain *NU14*. In the four immunized monkeys, one monkey acquired a partial infection with a positive urine culture on day 2. In the four control monkeys, all monkeys developed bladder infections (one partial).



DISCUSSION

Urinary tract infections are one of the most common infectious diseases. It is necessary to increase our knowledge of the pathogenesis of UTIs as a basis for prevention and for the establishment of new therapeutic and prophylactic strategies.

The attachment of *E. coli* to host cells is an important step in the initiation of UTI. The binding specificity of P-fimbriae has, to cryostat sections of tissues from different levels of the urinary tract, been described earlier. It has been shown that all outlining cell types in the human kidney as well as the epithelium and vessel wall of the urinary bladder can act as target sites in this adhesion (107). Furthermore, human uroepithelial cells, both voided and grown *in vitro*, has been used to study adhesion with *E. coli* (108, 109). The pathogenic role for P-fimbriae in pyelonephritis has also been demonstrated in the female Balb/c mouse model of UTI (110).

We have demonstrated P-fimbriated adhesion *in vitro* to viable human proximal tubular cell, as well as to two established bladder cell lines. The PagG class II positive *DS17* and *JR1* adhered avidly, while the two mutants lacking PapG class II did not adhere. Several clinical observations suggest that P-fimbriae contribute to the ability of *E. coli* strains to cause UTI, especially the more clinically severe forms, and that the strains lacking P-fimbriae are at disadvantage in the urinary tract (111). The isogenic mutant strains lacking the PapG class II adhesin were unable to adhere to urogenital cells in the present study. It has previously been shown that both the mutants and the wild type strain *DS17* are able to cause bladder infection in our monkey model. Epidemiological data have shown that first time cystitis is often caused by P-fimbriated strains (112). Thus, the PagG class II adhesin may also play a role in the establishment of bladder infections.

The flow cytometer examinations showed, in parallel, that cells incubated with the FITC-labelled wild type strains *DS17* and *JR1* exhibited a more extensive binding compared to the mutants lacking the PapG class II adhesin. However, renal cells incubated with FITC-labelled mutants showed slightly higher mean fluorescence intensity compared to cells unexposed to FITC-labelled bacteria. This indicates the presence of non-receptor mediated, P-fimbriae independent, binding of bacteria to renal cells. This is consistent with earlier investigations showing that non-specific physiochemical interactions also are involved in the adhesion process (113).

By introducing a digalactoside-containing carbohydrate-structure in the adhesion model, which competitively inhibited adhesion, we could further demonstrate the specificity for the PapG class II adhesin in the mediated attachment.

This *in vitro* model also provides the possibility to further study anti-adherence, inflammatory response and intracellular signal transduction following established bacterial adhesion.

The importance of immunity in the defence against recurrent UTIs is in many respects unclear (10, 114). Clinical experience suggests that recurrent infections in the urinary tract induce a protective immune response. It has been described how *E. coli* isolates can become phenotypically altered during episodes of recurrent UTI (26) or replaced by more uncommon bacteria. These findings indicate that a change of host defence may occur with recurrent *E. coli* cystitis. Immunity studies during cystitis have mainly focused on the antibody response and not on the protective effect. It is obvious that in the urinary tract immunoglobulines can be present at various levels (10, 115). The protective effect of these immunoglobulines remains unclear. However, in rats, both bladder and vaginal immunization were followed by a rapid immune response following subsequent bladder challenge with live *E. coli* (116-118).

In our primate infection model we were able to show that primary bladder infection caused by the wild type PapG class II positive strain *DS17* induced protection against subsequent rechallenge with both the PapG negative strain *DS17-8* and the PapG class III positive strain *DS17-1*, as well as with the heterologous wild type strain *JR1*. It was obvious that the PapG class II tip adhesin was necessary to induce protection.

Surprisingly, the protection also covered the mutant *DS17-8* lacking the tip adhesin. Our hypothesis is that the adherence mediated by the globoside binding PapG class II adhesin to uroepithelial cells creates an optimal milieu for the host response to react against other bacterial antigens such as LPS. The crucial role for the PapG class II adhesin may rather be to act as an adjuvant. The fact that neither *DS17-1* nor *DS17-8* induced any protective effect in the infection model strengthens this hypothesis.

Previous studies by other groups have shown that the capacity of primates to spontaneously clear *E. coli* cystitis correlates to both local and systemic immune response

against the infecting bacteria (75). In the present study, initial infection with the wild type *DS17* induced a systemic immune response with both IgA and IgG in serum in the majority of the monkeys. In urine, a local sIgA immune response was seen after an initial infection with *DS17* but not after infections with the two mutants. The correlation in urine between the antibody levels determined with the conjugates, anti-IgA and the anti-secretory component, indicates that much of the IgA was of the secretory type which is supposed to be produced locally.

It has been postulated that urinary sIgA may play a major role in the local defence mechanism of the human urinary tract (73). One possible mechanism may be that urinary sIgA inhibits bacterial colonization by lowering the bacterial adherence to the mucosa (74, 119).

In the present study, four of five monkeys showed a positive bacterial agglutination test in convalescent urine samples after *DS17* infection in contrast to neither of the two monkeys infected with *DS17-8*. In contrast, we failed to inhibit the haemagglutination test with *DS17* by adding convalescent samples of serum or urine after *DS17* infection, i.e. we could not block the PagG class II specific adhesion to globoside with our “high titer” samples. This finding indicates a biological activity in urine against antigens other than the adhesive PapG class II adhesin. These results in the present study provide further information on the complexity of host response in cystitis and they contribute to the understanding of the pathogenesis of lower urinary tract infections.

In the ambition to find a vaccine preventing recurrent cystitis, we focused on the Type 1-fimbriae, since the P-fimbriae are expressed in only the 50% of *E. coli* strains isolated from first time cystitis and thereafter in even less per centage (39). The Type 1-fimbriae are expressed in a vast majority of cystitis strains (43). Previous investigations in mice have shown that immunization with the FimH gave rise to protection against bladder infection caused by Type 1-fimbriated *E. coli* strains (98).

Our results demonstrate that a FimH vaccine induces protection against cystitis in a primate model. In the pathogenesis of UTIs, colonization of the vaginal mucosa precedes the bladder infection. We could show that functional antibodies of class IgG inhibited adherence of the Type 1-fimbriated strain *NUI4* to an established bladder cell line. The specific mechanism of the protective effect against induced cystitis is still unclear.

Detection of antibodies in urine were not performed in the present study. In the mice study mentioned above, urinary IgG against FimH were also detected in urine (98). The protective mechanisms may be complex, influenced by protective antibodies in both urine and vaginal secretion. The fact that the anatomy in female monkeys, in which the urethra terminates in the vagina, is different from women may influence the outcome of the immunization if the vaginal protection is of major importance. Further human studies will therefore be necessary to determine the efficacy of the FimH vaccine in humans.

CONCLUSIONS

- The PapG class II tip adhesin of P-fimbriated *E. coli* mediates specific adhesion to urogenital cells.
- A bladder infection with a PapG class II positive *E. coli* strain induces protection against subsequent challenge with homologous strain, while PapG class II negative mutants do not.
- The protective immune response following experimental bladder infections with a PapG class II positive strain correlates to levels of secretory IgA in urine.
- Immunization with a fragment of the FimC/H protein derived from *E. coli* Type 1-fimbriae mediates protection against cystitis caused by *E. coli* in a primate model.

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