MAJOR ARTICLE

Escherichia coli 83972 Expressing a P fimbriae Oligosaccharide Receptor Mimic Impairs Adhesion of Uropathogenic *E. coli*

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Urinary tract infections (UTIs) caused by uropathogenic *Escherichia coli* (UPEC) are a significant health concern, exacerbated by the rapid emergence of multidrug resistant strains refractory to antibiotic treatment. P fimbriae are strongly associated with upper urinary tract colonization due to specific binding to α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside receptors in the kidneys. Thus, inhibiting P-fimbrial adhesion may reduce the incidence of UPEC-mediated UTI. *E. coli* 83972 is an asymptomatic bacteriuria isolate successfully used as a prophylactic agent to prevent UTI in human studies. We constructed a recombinant *E. coli* 83972 strain displaying a surface-located oligosaccharide P fimbriae receptor mimic that bound to P-fimbriated *E. coli* producing any of the 3 PapG adhesin variants. The recombinant strain, *E. coli* 83972::*lgtCE*, impaired P fimbriae–mediated adhesion to human erythrocytes and kidney epithelial cells. Additionally, *E. coli* 83972::*lgtCE* impaired urine colonization by UPEC in a mouse UTI model, demonstrating its potential as a prophylactic agent to prevent UTI.

Urinary tract infections (UTIs) are among the most common infectious diseases of humans, with an estimated 150 million cases per year [1]. The recurrence rate is high, and infections frequently become chronic after multiple episodes. Bacterial interference using nonvirulent bacteria to prevent infection by pathogens is an alternative therapy for patients with chronic and recurrent UTIs that do not respond to antimicrobial treatment [2]. *Escherichia coli* 83972 is a clinical asymptomatic bacteriuria (ABU) isolate capable of long-term bladder colonization without causing disease symptoms and has been effectively used as a prophylactic agent for the prevention of UTI in studies involving humans [2–5]. *E. coli* 83972 does not adhere to host cells or induce a host inflammatory response because notable virulence determinants, including P, type 1, and F1C fimbriae are attenuated in this strain [6–8]. In clinical studies, patients deliberately colonized with *E. coli* 83972 by bladder inoculation did not develop systemic illness, had reduced frequency of symptomatic UTI, and reported a subjective increase in quality of life [2–5].

Uropathogenic *E. coli* (UPEC) is the most common etiological agent of UTI. UPEC cause >80% of UTIs, and in many cases these strains are resistant to multiple different antibiotics [9, 10]. The ability of UPEC to colonize the urinary tract and cause disease is strongly associated with the expression of fimbrial adhesins [11]. Adherence to the uroepithelium enables UPEC to resist the hydrodynamic forces of urine flow, trigger host and bacterial cell–signaling pathways, and establish infection. Among the characterized UPEC adhesins, P and type 1 fimbriae correlate with uropathogenesis and mediate binding to specific receptors within the urinary tract [12–15]. UPEC P fimbriae are most

Received 14 December 2011; accepted 14 May 2012; electronically published 7 August 2012.

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The Journal of Infectious Diseases 2012;206:1242–9

DOI: 10.1093/infdis/jis493

strongly associated with colonization of the upper urinary tract [14], and UPEC strains that cause pyelonephritis commonly encode multiple copies of P fimbriae gene clusters in their genome [16]. P fimbriae contribute to colonization by binding to the α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside receptor epitope in the globoseries of glycolipids found in human kidneys and on erythrocytes [17, 18]. P fimbriae recognize their receptors by virtue of the organelle tip–located adhesin PapG; 3 major classes of PapG adhesins have been characterized (PapGI, PapGII, and PapGIII), and these preferentially bind to different Galα[1 → 4]Galβ epitopes [17, 18]. P fimbriae contribute to uroepithelial cell adherence and induction of innate immune responses in animals and humans [8, 14, 19]. Therefore, inhibiting P-fimbrial adhesion may not only prevent urinary tract colonization by UPEC, but may also prevent the induction of inflammation in the kidney that can result in the acute pathology seen in cases of pyelonephritis.

Several studies have demonstrated that oligosaccharide receptor mimics inhibit the attachment of bacterial adhesins and toxins to host cells [20, 21]. Paton and colleagues developed a method to engineer recombinant bacteria expressing oligosaccharide receptor mimics on their cell surface, using heterologous galactosyltransferase genes [22]. This technology was used to create several probiotics, including a Shiga toxin (Stx) receptor mimic, a heat-labile enterotoxin receptor mimic, and a cholera toxin receptor mimic [20, 22]. We have used this technology to construct a recombinant *E. coli* 83972 strain displaying a P fimbriae receptor mimic on its surface. The galactosyltransferase genes *lgtC* and *lgtE* from *Neisseria meningitidis* and *Neisseria gonorrhoeae,* respectively, encoding synthesis of the oligosaccharide Galα[1 → 4]Galβ[1 → 4]Glc- (the known receptor for Stx), were inserted into the chromosome of *E. coli* 83972. We hypothesized that expression of the *lgtCE* genes would modify the naturally rough lipopolysaccharide (LPS) outer core of *E. coli* 83972 into a P fimbriae receptor mimic. Here, we assessed the ability of the recombinant strain *E. coli* 83972::*lgtCE* to bind to P-fimbriated *E. coli*, inhibit P fimbriae-mediated adhesion to human cells, and prevent colonization of the mouse urinary tract by P-fimbriated UPEC.

METHODS

Bacterial Strains, Plasmids, and Growth Conditions

Strains and plasmids used in this study are described in Table 1. P-fimbriated strains MS1250, MS1251, and MS1253 were constructed by transforming previously described plasmids encoding P-fimbrial genes into *E. coli* DH5α. Strain MS1250 contains plasmid pRHU845, which encodes the *pap* gene cluster from UPEC strain J96 (*papG*I allele) [27]. Strain MS1251 contains plasmid pPIL110-35, which encodes the *pap* gene cluster from UPEC strain AD110 (*papG*II allele) [28].

Table 1. Bacterial Strains and Plasmids used in this Study

Strain or plasmid	Description	Reference
Escherichia coli strains		
83972	Asymptomatic bacteriuria isolate	[3]
83972::*lgtCE*	*lgtCE-kan* genes inserted onto 83972 chromosome; Kan^R	This study
83972^{AMP}	83972 *attB*::*bla-rrnB*P1-*cfp*-T_0; Amp^R	[23]
CFT073	Pyelonephritis isolate	[24]
$CFT073^{CAM}$	CFT073*lacZ*::*cam*; Cam^R	[25]
MS1250	pRHU845 in DH5α	This study
MS1251	pPIL110-35 in DH5α	This study
MS1253	pJFK102 in DH5α	This study
OS56	MG1655*flu attB*::*bla*-$P_{A1/04/03}$-*gfp*mut3b*-T_0; Gfp^+	[26]
Plasmids		
pJCP-Gb_3	pK184 with stabilized *lgtC* and *lgtE*	[22]
pRHU845	*pap* allele I from UPEC J96 inserted into pACYC184	[27]
pPIL110-35	*pap* allele II from UPEC AD110 inserted into pACYC184	[28]
pJFK102	*pap* allele III from UPEC J96 inserted into pBR322	[29]
pBR322	Cloning vector	[30]
pKD4	Template plasmid for kanamycin resistance gene	[31]
pKD46	Helper plasmid encoding λ Red recombinase	[31]
pCP20	Helper plasmid encoding FLP recombinase	[31]
pRW13	*lgtCE* from pJCP-Gb_3 inserted into pBR322	This study
pRW16	pKD4 kanamycin resistance gene inserted into pRW13	This study
pmCherry	Vector encoding mCherry fluorescent protein	Clontech

Strain MS1253 contains plasmid pJFK102, which encodes the *prs* gene cluster from UPEC J96 (*papG*III allele) [29]. Strains were routinely grown at 37°C on solid or in liquid Luria-Bertani (LB) medium. LB agar plates were supplemented with appropriate antibiotics in the following final concentrations: 50 μg mL^{-1} ampicillin, 50 μg mL^{-1} kanamycin, 15 μg mL^{-1} tetracycline, and 15 μg mL^{-1} chloramphenicol.

Construction of the Recombinant Strain *E. coli* 83972::*lgtCE*

To construct *E. coli* 83972::*lgtCE*, the *lgtCE* genes from pJCP-Gb_3 [22] were integrated into the chromosome of *E. coli* 83972. First, the *lgtCE* genes from plasmid pJCP-Gb_3 were amplified by polymerase chain reaction (PCR) using primers 1931 (5′-TGACCATGATTACGAATTCG) and 1932 (5′-GC GCCGTCGACTAAGTTGGGTAACGCCAGGG). The PCR product was digested with *Sal*I and ligated into *Eco*RV/*Sal*I-digested pBR322 downstream of the constitutive tetracycline

resistance gene promoter to create plasmid pRW13. Next, the kanamycin resistance gene (*kan*) and FLP recognition target (FRT) sites were amplified from pKD4, using primers 2060 (5′-GCGGCGTCGACGTGTAGGCTGGAGCTGCTTC) and 2061 (5′-CCCGCGTCGACCATATGAATATCCTCCTTAG). The PCR product was digested with *Sal*I and ligated into *Sal*I-digested pRW13 downstream of the *lgtCE* genes to create pRW16. The λ Red recombination method was used as previously described [31] to insert the *lgtCE-kan* genes from pRW16 into *E. coli* 83972 within the nonfunctional type 1 fimbrial gene cluster (ie, between *fimD* and *fimH*). The *lgtCE-kan* genes were PCR amplified from pRW16, using primers 2063 (5′-ATGGCAGCATATCAATACCTGGCTTGAGCGA GACATAATTCCGTTACGTTACATATTTGAATGTATTTAG) and 2064 (5′-TACTGCTCCTAACGATACCGTGTTATTCGC TGGAATAATCGTACCGTTGCTCTCAAGGGCATCGGTCG AC), which contain 50-base-pair ends (5′) complementary to the *fim* gene cluster allowing homologous recombination. The PCR product was transformed into *E. coli* 83972(pKD46) to construct the recombinant strain *E. coli* 83972*fim'*::*lgtCE* (referred to as 83972::*lgtCE*). *E. coli* 83972::*lgtCE* was confirmed by PCR and DNA sequencing with primers 97 (5′-CAA TATTCGTCATACTGTCTGG), 1924 (5′-CGCAATCGGCAA TGTATTCG), 78 (5′-CGGCCACAGTCGATGAATCC), and 331 (5′-GCAGTCACCTGCCCTCCGGTA).

Stx Adsorption/Neutralization Assay

Stx neutralization was assayed as previously described, using 10 μg mL^{-1} purified Stx2 or a lysate of *E. coli* JM109:pJCP525 as a source of Stx1 [22].

Agglutination Assays

E. coli strains 83972, 83972(pJCP-Gb_3) and 83972::*lgtCE* were grown in LB medium shaking at 37°C for 16 hours. Strain 83972(pJCP-Gb_3) was induced for *lgtCE* expression, using 1 mM IPTG. P-fimbriated *E. coli* strains MS1250, MS1251, and MS1253 and UPEC strain CFT073 were grown on LB agar plates at 37°C for 16 hours and subsequently resuspended in phosphate-buffered saline. Cell suspensions were standardized to an optical density at 600 nm of 1.0; a volume of 10 μL of an *E. coli* 83972 strain was mixed with 10 μL of a P-fimbriated strain on glass slides, and the presence or absence of visible agglutination was recorded. Agglutination between fluorescently labeled strains OS56(pPIL110-35), 83972(pmCherry), and 83972::*lgtCE*(pmCherry) was performed as described above and observed using an LSM 510 META confocal microscope (Zeiss). Hemagglutination using human type A red blood cells (RBCs) was assessed as previously described [32]. Strains were grown as for mixed agglutinations, except tryptic soy agar plates were used to optimize P-fimbrial expression [33]. *E. coli* 83972 or 83972::*lgtCE* were mixed with P-fimbriated strains MS1251 and CFT073 in 1:1, 5:1, or 10:1 ratios before 20 μL of the combined strains were mixed with 10 μL of RBCs. Hemagglutination was assessed visibly for up to 5 minutes, with the time taken to cause agglutination recorded.

Kidney Epithelial Cell Adhesion Assays

Human A498 kidney epithelial cells were grown in Advanced MEM supplemented with 10% heat-inactivated fetal bovine serum (Gibco). Epithelial cells were seeded into 24-well cell culture plates (Corning) at a concentration of 2×10^5 cells per well and incubated overnight at 37°C in 5% CO_2. Monolayers were washed 5 times with 10 mM Tris before inoculation with bacteria. Antibiotic-resistant strains *E. coli* 83972^{AMP} and $CFT073^{CAM}$ were used as the wild-type strains to facilitate plating on selective antibiotic plates; these strains have been described previously and possess growth rates that are identical to those of *E. coli* 83972 and CFT073, respectively [23, 25]. Strains 83972^{AMP} or 83972::*lgtCE* were mixed with MS1251 or $CFT073^{CAM}$ in a 10:1 ratio for 5 minutes before the epithelial cell monolayers were inoculated. Inoculated monolayers were incubated at 37°C in 5% CO_2 for 1 hour before washing 5 times with 10 mM Tris-HCl, pH 7.4. Monolayers were lysed with 0.01% Triton-X 100 in distilled water, and quantitative colony counts were performed on LB agar plates containing appropriate antibiotics to enable discrimination between strains.

Mouse Model of UTI

Female C57BL/6 mice (8–10 weeks) were purchased from the Animal Resources Centre, Australia. The mouse model of UTI was performed as previously described [34]. Mice were inoculated with a mixture of 1×10^6 colony-forming units (CFU) of *E. coli* $CFT073^{CAM}$ and 1×10^8 CFU of *E. coli* 83972^{AMP} or 83972::*lgtCE*, grown for 20 hours in LB medium. Bacterial strains were mixed for 30 minutes before inoculation into the mice. Urine was collected at 24 hours after inoculation for quantitative colony counts. Mice were subsequently euthanized, and the bladders and kidneys were excised and processed for colony counts. The strains were differentiated by resistance to chloramphenicol (*E. coli* $CFT073^{CAM}$), ampicillin (83972^{AMP}), and kanamycin (83972::*lgtCE*). Animal experimentation was approved by the animal ethics committee of Griffith University (number MSC/14/08AEC).

Statistical Analysis

Differences in hemagglutination times and bacterial adherence to kidney epithelial cells (in CFU per mL) were tested using unpaired 2-sample *t* tests with Minitab 14 statistical software. Differences in mouse UTI colonization by *E. coli* $CFT073^{CAM}$ (CFU/mL or CFU/0.1 g) were tested using the nonparametric Mann–Whitney *U* test. The level of statistical significance was set at $P < .05$.

RESULTS

Construction of the Recombinant Strain *E. coli* 83972::*lgtCE* Expressing a P Fimbriae Receptor Mimic

Plasmid pJCP-Gb_3 contains the galactosyltransferase *lgtCE* genes that direct synthesis of the oligosaccharide Galα[1 → 4]Galβ[1 → 4]Glc- at the distal end of the *E. coli* LPS core, both in strains such as CWG308, which has a *waaO* mutation that truncates the LPS core so that it terminates in Glc [22], as well as in other strains of *E. coli* and *Salmonella enterica* with wild-type LPS core regions, wherein the overexpressed heterologous transferases outcompete the endogenous enzymes for the Glc-terminating LPS core intermediate [35]. To confirm appropriate expression of the Galα[1 → 4]Galβ[1 → 4]Glc- epitope in the 83972 background, *E. coli* 83972(pJCP-Gb_3) suspensions were tested for their capacity to bind and neutralize Stx1 and Stx2. In both cases, >99% of toxin was neutralized. LPS extracts were also analyzed by polyacrylamide gel electrophoresis and silver staining. Both toxin neutralization capacity and LPS profile were indistinguishable from that previously reported [22] for *E. coli* CWG308:pJCP-Gb_3 (data not shown). However, to ensure stable expression of the Galα[1 → 4]Galβ[1 → 4]Glc-trisaccharide, a strategy was devised to integrate the *lgtCE* genes from pJCP-Gb_3 into the chromosome of *E. coli* 83972 (Figure 1). This resulted in the construction of the recombinant strain *E. coli* 83972*fim'*::*lgtCE* (referred to as 83972::*lgtCE*). We hypothesized that expression of the Galα[1 → 4]Galβ[1 → 4]Glc- trisaccharide should result in binding of *E. coli* 83972::*lgtCE* to P-fimbriated *E. coli*.

E. coli 83972::*lgtCE* Agglutinates P-fimbriated *E. coli*

We examined whether *E. coli* 83972::*lgtCE* expressing Galα[1 → 4]Galβ[1 → 4]Glc- on the cell surface could agglutinate P-fimbriated *E. coli*. *E. coli* strains 83972 or 83972::*lgtCE* were mixed 1:1 with P-fimbriated strains MS1250 (*papG*I allele), MS1251 (*papG*II allele), MS1253 (*papG*III allele), or UPEC CFT073 (which contains 2 chromosomally encoded *pap* gene clusters—both *papG*II alleles) and monitored for visible agglutination. *E. coli* 83972::*lgtCE* agglutinated all PapG variant P-fimbriated *E. coli* strains. As expected, *E. coli* 83972 did not bind to any of the P-fimbriated strains. To visualize the specific agglutination of *E. coli* 83972::*lgtCE* with a P-fimbriated strain, we used fluorescently labeled strains in combination with confocal scanning laser microscopy. First, plasmid pPIL110-35 (*papG*II) was transformed into *E. coli* OS56 to generate a P-fimbriated, green fluorescent strain OS56(pPIL110-35). Plasmid pmCherry was transformed into both *E. coli* 83972 and 83972::*lgtCE* to obtain fluorescently tagged strains. Strains were mixed 1:1 and examined for agglutination. As expected, no agglutination was observed for *E.*

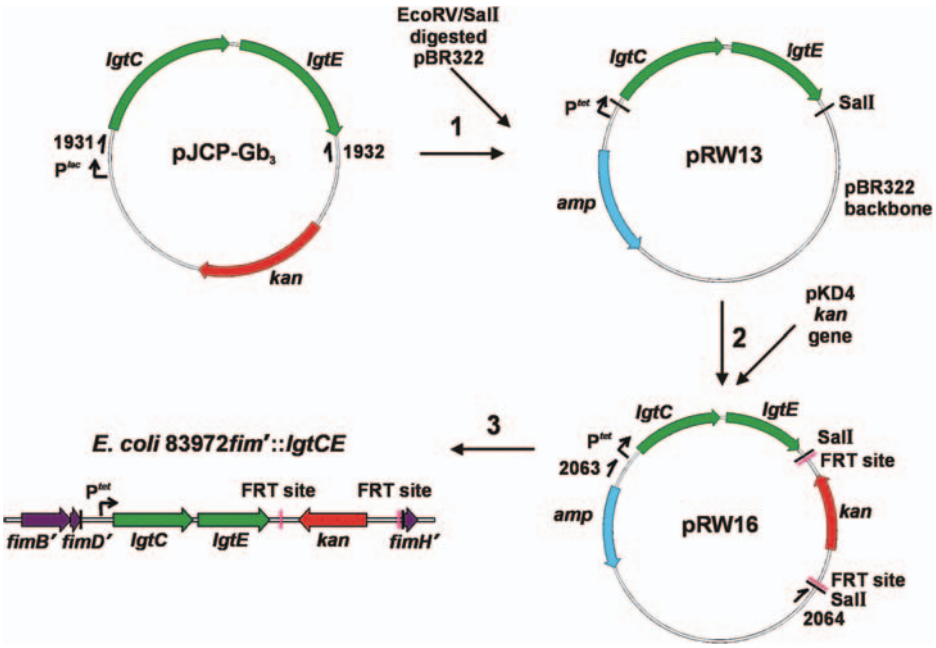

Figure 1. Construction of *Escherichia coli* 83972*fim'*::*lgtCE*. (1) The *lgtCE* genes from pJCP-Gb_3 were amplified by polymerase chain reaction (PCR), using primers 1931 and 1932, and ligated into plasmid pBR322 to construct pRW13. (2) The kanamycin resistance gene (*kan*) was PCR amplified from pKD4 and ligated to SalI digested pRW13 to create pRW16. (3) The *lgtCE-kan* genes from pRW16 were PCR amplified using primers 2063 and 2064 and inserted into the incomplete *E. coli* 83972 type 1 fimbrial gene cluster by λ Red-mediated homologous recombination. Black lines indicate insertion sites; pink lines indicate FRT sites. P^{lac} and P^{tet} are the *lac* promoter and tetracycline resistance promoter, respectively. Fimbrial genes *fimB'*, *fimD'*, and *fimH'* are not intact.

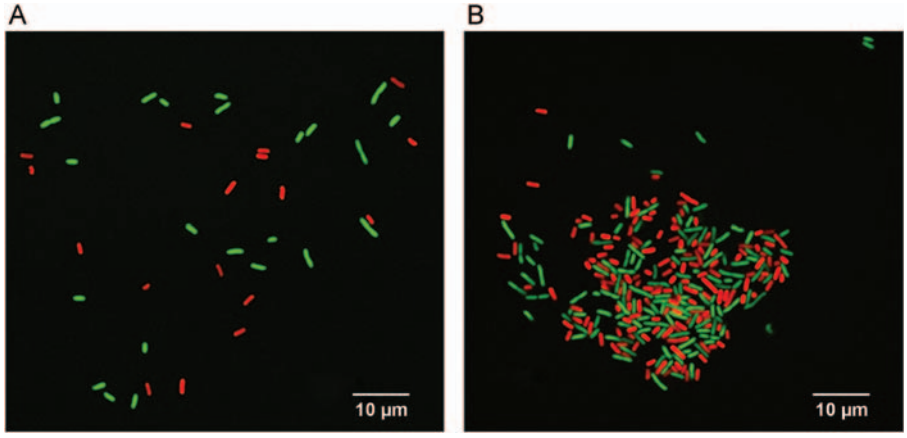

Figure 2. Bacterial agglutination of *Escherichia coli* 83972::*lgtCE* with P-fimbriated *E. coli*. Confocal scanning laser microscopy (×1000 magnification) of green fluorescent, P-fimbriated *E. coli* OS56(pPIL110–35) mixed 1:1 with pmCherry fluorescent (*A*) 83972(pmCherry) and (*B*) 83972::*lgtCE*(pmCherry).

coli 83972(pmCherry) mixed with OS56(pPIL110-35) (Figure 2). However, agglutination of green and pmCherry fluorescent bacteria was observed for *E. coli* 83972::*lgtCE* (pmCherry) mixed with OS56(pPIL110-35). Therefore, *E. coli* 83972::*lgtCE* stably expresses the Galα[1 → 4]Galβ[1 → 4]Glc-P fimbriae receptor mimic and specifically binds to P-fimbriated *E. coli* expressing any of the 3 PapG variants.

E. coli 83972::*lgtCE* Impairs Binding of P-fimbriated *E. coli* to Human RBCs

P fimbriae adhere to and agglutinate human RBCs via specific interaction with Galα[1 → 4]Galβ saccharides [17]. Hemagglutination assays were performed to assess whether *E. coli* 83972::*lgtCE* could inhibit the ability of P-fimbriated *E. coli* to agglutinate RBCs. We tested the ability of MS1251 (*papG*II) and UPEC CFT073 to bind to RBCs in the presence of *E. coli* 83972 or 83972::*lgtCE*. Wild-type *E. coli* 83972 had no effect on MS1251 or CFT073 hemagglutination times when mixed 1:1, 5:1, or 10:1 with the P-fimbriated strains (Figure 3). However, *E. coli* 83972::*lgtCE* significantly increased the MS1251 hemagglutination times when mixed 5:1 or 10:1, compared with *E. coli* 83972 ($P < .05$). A more pronounced effect was seen for *E. coli* CFT073, in which a 1:1 ratio of 83972::*lgtCE* and CFT073 was enough to significantly increase the hemagglutination time, compared with *E. coli* 83972 ($P < .05$). Additionally, at a 10:1 ratio of 83972::*lgtCE* to CFT073, there was no visible hemagglutination after 5 minutes. Thus, *E. coli* 83972::*lgtCE* interferes with the binding of P-fimbriated bacteria to human cells.

E. coli 83972::*lgtCE* Impairs Binding of P-fimbriated *E. coli* to Human Kidney Cells

P fimbriae adhere to human kidney epithelial cells, and this is strongly associated with colonization of the upper urinary tract [19]. To further demonstrate that *E. coli* 83972::*lgtCE* interferes with the function of P fimbriae as an adhesin, quantitative adhesion assays using A498 human kidney cells were performed. We tested the ability of P-fimbriated strains MS1251 (*papG*II) and CFT073^{CAM} to adhere to kidney cells in the presence of *E. coli* 83972^{AMP} or 83972::*lgtCE*. Strain MS1251 adherence to kidney cells was 92% lower when mixed with 83972::*lgtCE* than when mixed with strain 83972^{AMP} ($P < .05$; Figure 4). Additionally, strain CFT073^{CAM} adherence to kidney cells was 72% lower in the presence of 83972::*lgtCE*, compared with 83972^{AMP} ($P < .05$). Therefore, *E. coli* 83972::*lgtCE* impaired the kidney epithelial cell adhesion of P-fimbriated *E. coli*, including UPEC strain CFT073^{CAM}.

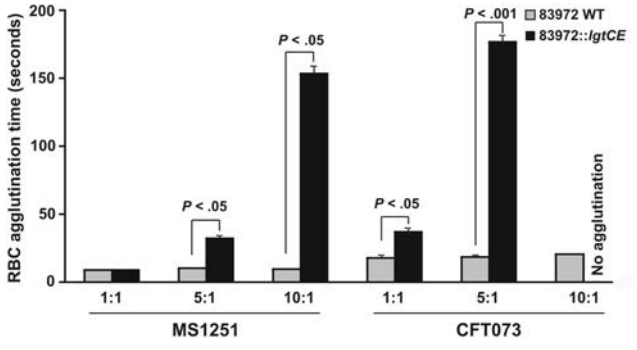

Figure 3. Hemagglutination by P-fimbriated *Escherichia coli* MS1251 or CFT073 in the presence of *E. coli* 83972 or 83972::*lgtCE*. Time (seconds) taken for strains to visibly agglutinate with human RBCs. Results are expressed as the average of triplicate assays + SD. The presence of *E. coli* 83972::*lgtCE* significantly increased the time to hemagglutination, compared with *E. coli* 83972, when mixed 5:1 and 10:1 with MS1251 or 1:1 and 5:1 with CFT073 ($P < .05$ by unpaired 2-sample *t* tests). For *E. coli* 83972::*lgtCE* mixed 10:1 with CFT073, there was no visible red blood cell agglutination after 5 minutes.

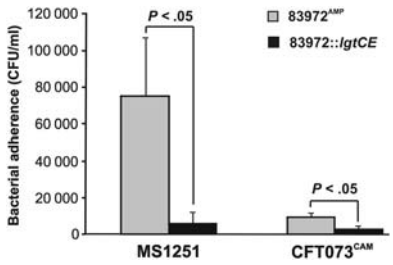

Figure 4. Kidney epithelial cell adhesion of P-fimbriated *E. coli* in the presence of *E. coli* 83972^{AMP} or 83972::*lgtCE*. A498 kidney cell monolayers were inoculated with *E. coli* MS1251 or CFT073^{CAM} mixed with *E. coli* 83972^{AMP} (grey bars) or 83972::*lgtCE* (black bars). Adherent colony-forming units (CFU) per milliliter are shown for MS1251 and CFT073^{CAM}. Results are expressed as the average of at least triplicates + SD. Kidney cell adhesion by MS1251 and CFT073 was significantly impaired in the presence *E. coli* 83972::*lgtCE* compared to 83972^{AMP} ($P < .05$ by unpaired 2-sample *t* tests).

E. coli 83972::*lgtCE* Impairs UPEC Colonization in a Mouse UTI Model

A mouse UTI model was used to determine whether *E. coli* 83972::*lgtCE* could inhibit urinary tract colonization by P-fimbriated UPEC. *E. coli* 83972^{AMP} or 83972::*lgtCE* were mixed 100:1 with UPEC strain CFT073^{CAM} and inoculated into the bladder of C57BL/6 mice. The median number of *E. coli* CFT073^{CAM} recovered from the urine was significantly lower in mice that were challenged with a mixed inoculum containing *E. coli* 83972::*lgtCE*, compared with those that received a mixed inoculum containing 83972^{AMP} ($P = .014$; Figure 5). There was no difference in the median number of *E. coli* CFT073^{CAM} recovered from the bladder or kidneys when mixed with *E. coli* 83972::*lgtCE* or when mixed with 83972^{AMP} ($P = .175$ and $P = .456$, respectively; data not shown). Thus, *E. coli* 83972::*lgtCE* expressing a P fimbriae oligosaccharide receptor mimic significantly prevented urine colonization by UPEC in the mouse UTI model.

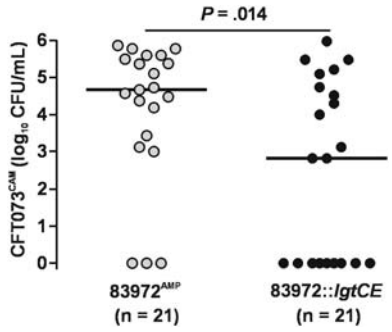

Figure 5. Mouse urinary tract colonization by *E. coli* CFT073^{CAM} in the presence of *E. coli* 83972^{AMP} or 83972::*lgtCE*. C57BL/6 mice were transurethrally inoculated with a mixture of 1×10^6 colony-forming units (CFU) of CFT073^{CAM} and 1×10^8 CFU of 83972^{AMP} (n = 21) or 1×10^8 CFU of 83972::*lgtCE* (n = 21). *E. coli* CFT073^{CAM} $\log_{10}$ CFU per milliliter of urine is shown. Bars represent median values. The median number of *E. coli* CFT073^{CAM} recovered from the urine was significantly lower when mixed with *E. coli* 83972::*lgtCE* than when mixed with 83972^{AMP} ($P = .014$ by the Mann–Whitney *U* test).

DISCUSSION

In this study, we successfully engineered an *E. coli* 83972 strain that stably expresses a P fimbriae receptor mimic on the bacterial surface. We then assessed the ability of *E. coli* 83972::*lgtCE* to bind to P-fimbriated bacteria and to inhibit adhesion of P-fimbriated bacteria to host cells. *E. coli* 83972::*lgtCE* bound to *E. coli* strains expressing all 3 major classes of PapG adhesins, which preferentially bind to different Galα[1 → 4]Galβ glycolipid isoreceptors found on different host tissues [17, 18]. The P fimbriae receptor mimic Galα[1 → 4]Galβ[1 → 4]Glc- used in our study mimics globotriaosylceramide (Galα[1 → 4]Galβ[1 → 4]Glc-ceramide; Gb_3) found on human uroepithelial cells [17, 18]. The binding of *E. coli* 83972::*lgtCE* to PapGII is clinically relevant because *papG*II is the most highly prevalent allele among clinical UPEC isolates and is associated with human pyelonephritis and bacteremia [36, 37]. The finding that *E. coli* 83972::*lgtCE* bound to all 3 classes of PapG adhesins suggests it may be effective against all P-fimbriated UPEC.

E. coli 83972::*lgtCE* also impaired adhesion of P-fimbriated bacteria to both human erythrocytes and human kidney epithelial cells. Adherence of both MS1251 (*papG*II) and CFT073 to human cells was impaired by *E. coli* 83972::*lgtCE*, with differences in effectiveness most likely due to the different levels of P-fimbrial expression between these strains. Previous studies with UPEC strain CFT073 have demonstrated that even under optimal conditions for P fimbriae expression, <20% of the bacterial population will be P fimbriated [38, 39]. Thus, we demonstrated that *E. coli* 83972::*lgtCE* prevents adhesion of P-fimbriated *E. coli*, including the wild-type UPEC strain CFT073, to host cells by competitive inhibition. We also tested whether *E. coli* 83972::*lgtCE* could prevent urinary tract colonization by P-fimbriated *E. coli*, using a mouse UTI model. In a mixed infection assay, the number of *E. coli* CFT073 cells recovered from the urine of infected mice was significantly lower in mice inoculated with *E. coli* 83972::*lgtCE* as compared to 83972^{AMP}. *E. coli* 83972 asymptomatically colonizes the urine of humans but cannot ascend to the kidneys [40]. The finding that *E. coli* 83972::*lgtCE* impairs colonization of the urine by a P-fimbriated strain suggests it may be effective at preventing high-grade bacteriuria and subsequent kidney ascension by UPEC in humans.

Oligosaccharide receptor mimics are unlikely to exhibit toxicity, are poorly immunogenic, and would not be expected to

promote resistance [21, 41]. Several previous studies have examined the ability of both natural and synthetic receptor mimics to inhibit P fimbriae–mediated adhesion [42–47]. Human milk oligosaccharides act as anti-adhesives against P-fimbriated UPEC, and digalactoside-containing glycoproteins from pigeon and dove eggs reduced kidney infection by P-fimbriated *E. coli* in a mouse UTI model [42, 44]. Globotriose, mimicking Gb_3, inhibited and reversed binding of a P-fimbriated *E. coli* strain to human RBCs and decreased colonization of the mouse urinary tract [43]. Globotetraose, mimicking Gb_4, also decreased adhesion of a P-fimbriated *E. coli* strain to exfoliated human uroepithelial cells and decreased colonization of the mouse urinary tract [47]. However, synthetic oligosaccharide receptor mimics have not yet been used successfully in patients to prevent or treat infections.

Recombinant bacteria expressing oligosaccharide receptor mimics have several advantages over free, soluble oligosaccharides, which often need to be in a specific multivalent conformation, can be expensive and may be rapidly digested if administered orally [20, 21]. Indeed, the Stx receptor mimic has a binding capacity 10 000 times that of the synthetic Stx absorbent Synsorb Pk, which was used safely in humans but failed to prevent disease in phase II clinical trials [22, 41, 48]. *E. coli* 83972 is well maintained in the bladder, does not adhere to the uroepithelium or induce immune responses, outcompetes UPEC, and has been successfully used in patients to reduce symptomatic UTI [40]. Phase I and II clinical trials with *E. coli* 83972 have been performed in Sweden and the United States, with additional trials planned [2–5]. The epitope Galα[1 → 4] Galβ[1 → 4] Glc- occurs naturally on human cells, including uroepithelial cells, and was found to be safe for administration to children as part of Synsorb Pk [48]. Therefore, *E. coli* 83972::*lgtCE* expressing the same epitope is likely to be safe in patients. Using plasmid pCP20, we have now removed the kanamycin resistance cassette from *E. coli* 83972::*lgtCE*, resulting in the creation of an *E. coli* 83972::*lgtCE* strain with no antibiotic resistance marker that may be a more viable option for use as a probiotic. We predict that *E. coli* 83972::*lgtCE*, like wild-type *E. coli* 83972, would be most beneficial for patients highly susceptible to UTI, such as those individuals with a neurogenic bladder or catheterized patients. Overall, the recombinant *E. coli* receptor mimic strain described here may represent an alternative prophylactic treatment for patients with chronic or recurrent UTIs, a desperately needed alternative given the rapid emergence of multidrug-resistant UTI strains refractive to current treatment regimens.

Notes

Financial support. This work was supported by grants from the Australian National Health and Medical Research Council (569676 and 631654). M. A. S. and G. C. U. are supported by ARC Future Fellowships (FT100100662 and FT110101048, respectively). J. C. P. is a NHMRC Australia Fellow, and A. W. P. is an ARC DORA Fellow.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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