Vaccination with FimH Adhesin Protects Cynomolgus Monkeys from Colonization and Infection by Uropathogenic *Escherichia coli*

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*Escherichia coli* FimH adhesin mediates binding to the bladder mucosa. In mice, a FimH vaccine protects against bacterial challenge. In this study, 4 monkeys were inoculated with 100 μg of FimCH adhesin-chaperone complex mixed with MF59 adjuvant, and 4 monkeys were given adjuvant only intramuscularly. After 2 doses (day 0 and week 4), a booster at 48 weeks elicited a strong IgG antibody response to FimH in the vaccinated monkeys. All 8 monkeys were challenged with 1 mL of 10⁸ *E. coli* cystitis isolate NU14. Three of the 4 vaccinated monkeys were protected from bacteruria and pyuria; all control monkeys were infected. These findings suggest that a vaccine based on the FimH adhesin of *E. coli* type 1 pili may have utility in preventing cystitis in humans.

Acute urinary tract infections (UTIs) are among the most common disorders prompting medical evaluation. Forty percent of adult women in the United States experience at least one UTI sometime during their lifetime, with an annual incidence of 0.5%–0.7% in persons at highest risk [1]. This results in ~7 million office visits each year with an estimated annual health care cost >$1 billion [2]. *Escherichia coli* are the main causative agents of UTIs [3]. One method of eliminating acute UTIs is the use of regular or intermittent antimicrobial prophylaxis. Concern about the emergence of antibiotic-resistant bacterial strains limits the long-term feasibility of this approach [4].

Recent in vivo studies in mice demonstrated that colonization of the bladder by *E. coli* requires FimH [5, 6], the adhesin on type 1 pili, and immunization with FimH results in significant protection against challenge [5]. In vitro studies have shown that type 1 pili are important for attachment of *E. coli* to de-squamated epithelial cells from infected humans [7] and induce proinflammatory cytokine production by epithelial cells [6]. Anti-FimH antibody blocks in vitro attachment of *E. coli* to bladder cells and the concomitant inflammatory response [5]. Thus, blocking colonization of the bladder by inhibiting type 1 pilus-mediated binding to uroepithelium may be a strategy to block bladder infections.

**Methods**

**Bacterial strains, vaccine preparation, and reagents.** NU14 *E. coli* were isolated from a patient with recurrent cystitis [8]. A complex of ~52 kDa composed of the periplasmic chaperones FimC (22.8 kDa) and FimH (~29.1 kDa) in a 1 : 1 equimolar ratio was expressed in *E. coli* K12 strain C600 extracted from the periplasm and was purified to >99% purity by previously published methods [9]. The FimH T3 protein used in ELISAs represents a histidine-tagged fusion protein composed of the first 165 of a total of 279 aa of the mature FimH protein. The surfactant-stabilized emulsion adjuvant MF59 [10] (Chiron, Emeryville, CA), which was shown to be a potent and safe adjuvant in human subjects with several vaccines, is an oil-in-water formulation that contains surfactants (Tween 20 and Span 85) along with metabolizable oil (squalene) emulsified under high-pressure conditions.

**Immunization, bladder infection, and sampling of monkeys.** Cynomolgus monkeys (*Macaca fascicularis*, healthy adult females) were bred in China (Shunde Guandeng Experimental Farm) and transported to the Karolinska Institute primate center (Stockholm) at age ~4 years. Monkeys received either 100 μg of FimCH in MF59 adjuvant at a 1 : 1 ratio (immunized group) or MF59 plus diluent (control group) at weeks 0, 4, and 48. This immunization...
schedule was based on prior studies in mice with the MF59 adjuvant (unpublished data). Each 1-mL injection (total volume) was administered intramuscularly into the thigh; legs were alternated with each inoculation. Serum samples were collected once a month for assessment of immune responses. Vaginal wash and serum samples were collected before and after the last boost (weeks 47 and 50). Bladder infection was induced by inoculation of a bacterial suspension (1 mL, \(10^7\) cfu/mL) via urethral catheter; this is below the volumetric capacity of the bladder (void volume, >50 mL). Bacteria were grown under conditions to maximize type 1 pilus expression (see table 1). Samples were collected by suprapubic bladder aspiration twice a week until 2 successive negative cultures were obtained. At 20–30 min before bladder aspiration, the monkeys were hydrated with \(>50\) mL of lukewarm saline administered subcutaneously for optimal diuresis. Persisting infection after \(\sim 2.5\) weeks was eliminated through 1-time intramuscular injection of 5 mg of ciprofloxacin (Bayer, Leverkusen, Germany). All experiments were done under ketamine and midazolam anesthesia.

Cultivation of coliform bacteria in feces and determination of biochemical profile of fecal samples. A sterile cotton applicator stick was inserted 2–3 cm into the rectum of each monkey once a month throughout the year-long study. The swab was stirred into 1 mL of PBS, and the fecal suspension was cultivated in serial dilutions overnight at 37°C on cystine-lactose-electrolyte-deficient–agar plates (Oxoid, Basingstoke, UK). We counted the number of coliforms recovered from the swab into 1 mL of PBS. An automated system for biochemical analysis (Phene Plate [PhP system]; BioSys Inova, Stockholm) was used to identify biochemical phenotypes (BPTs) of coliforms in feces and to compare the diversity of bacteria in different samples. Reagents used in the PhP plates and the method for comparison of bacterial populations have been described [12]. In brief, each microplate contains 8 parallel sets of 12 dehydrated reagents, specifically chosen to differentiate coliform bacteria. In all, 768 samples (8 coliform isolates from each of 12 fecal samples \(\times 8\) monkeys) were subjected to biochemical typing (BP). BP provided information about which coliform strains colonized the gut of each monkey at each sampling point.

We set an identity level of 0.975 based on reproducibility of the system as described elsewhere [12]. Isolates showing correlation coefficients higher than the identity level were assigned to the same BPTs. The phenotypic similarity between different bacterial populations in 2 samples was calculated as the population similarity (Sp) coefficient as described elsewhere [12].

Results

Bladder infections with type 1–piliated \(E\. coli\). Before testing the FimH vaccine in the primate model, we demonstrated that \(E\. coli\) expressing type 1 pili could colonize monkey bladders. NU14 bacteria (80%–90% type 1 +) persistently infected monkey bladders for 4–7 days after challenge when administered in doses of \(10^7\) or \(10^8\) bacteria. Two monkeys were tested for each dose, and the number of bacteria recovered in urine samples was similar for both doses, ranging from \(\sim 5 \times 10^7\) to \(5 \times 10^6\) cfu/mL on days 2, 4, and 7 after challenge. One monkey challenged with the \(10^8\) dose cleared the infection by day 7. Monkeys inoculated with comparable doses of an isogenic fimH knockout strain [5] had no bacteria in their urine 2 days after inoculation, the first time point tested, and throughout the 2-week study course. The 4 monkeys used in this colonization study were not included in the vaccination and challenge studies described below.

Antibody response to FimCH immunization. Systemic IgG antibody levels to FimH were sustained throughout the first 47 weeks of the study in all 4 FimCH-vaccinated monkeys (figure 1A). Significant booster responses were seen in all 4 monkeys after the final vaccination at 48 weeks; the increase in titers ranged from 32-fold (monkey 5) to 256-fold (monkey 8). Control monkeys exhibited no antibodies to FimH at a 1:100 dilution of antisera, the limit of detection of the assay.

IgG antibodies to FimH were also detected in vaginal wash

Table 1. Colonization of monkey bladders and inflammatory responses following challenge with \(10^8\) \(Escherichia coli\) NU14.

<table>
<thead>
<tr>
<th>Day</th>
<th>No. of monkeys colonized</th>
<th>Inflammatory response</th>
<th>No. of monkeys colonized</th>
<th>Inflammatory response</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Monkey 5</td>
<td>Monkey 6</td>
<td>Monkey 7</td>
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<tr>
<td>2</td>
<td>1/4</td>
<td>++</td>
<td>0</td>
<td>0</td>
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<tr>
<td>4</td>
<td>0/4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0/4</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>0/4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
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</table>

NOTE. Bacteria were cultivated overnight on LB agar (Duchefa, Harlem, The Netherlands). Expression of type 1 pili was induced by 2 48-h serial passages in static brain-heart infusion broth (Difco Labs, Detroit) culture at 37°C. Before infection, expression of type 1 pili was quantitated by titration of bacterial suspension and mixing of equal volumes of 5% yeast cells (\(Saccharomyces cerevisiae\)) and bacteria in microtiter cells. Bacterial suspensions showed agglutination titer of \(\geq 30-60\). For quantitative estimation of FimH directly in broth cultures used for bladder inoculation, slightly modified IFA (method from [11]) was used. Some \(\sim 80\%–90\%\) of \(E\. coli\) NU14 expressed type 1 pili before inoculation. Monkeys were challenged with \(10^8\) cfu/mL based preliminary challenge studies in monkeys, which showed that \(\geq 10^7\) bacteria would constitute appropriate infectious dose. Bacteria in urine samples from days 2, 4, 7, and 12 after challenge were counted by streaking 1 mL of PBS. An automated system for biochemical analysis (Phene Plate [PhP system]; BioSys Inova, Stockholm) was used to identify biochemical phenotypes (BPTs) of coliforms in feces and to compare the diversity of bacteria in different samples. Reagents used in the PhP plates and the method for comparison of bacterial populations have been described [12]. In brief, each microplate contains 8 parallel sets of 12 dehydrated reagents, specifically chosen to differentiate coliform bacteria. In all, 768 samples (8 coliform isolates from each of 12 fecal samples \(\times 8\) monkeys) were subjected to biochemical typing (BP). BP provided information about which coliform strains colonized the gut of each monkey at each sampling point.

*a* Monkeys were monitored at indicated times for recoverable bacteria in urine. Monkey 8 was not included in these studies because it died of unrelated causes. Bacteria were identified as \(E\. coli\) based on colony morphology, Gram stain, and biochemical reactions.

*b* Leukocytes were counted to establish no. of cfu/mL urine. Urine specimen was considered positive when it contained \(\geq 10^5\) cfu/mL. To establish that inoculating strain was recovered in urine, urinary bacteria were analyzed on prepared microplates for rapid typing of coli form bacteria using PhenePlate system [12].

° Ciprofloxacin was used to clear infection in this monkey.
Figure 1. IgG titers to FimH in primate sera and vaginal wash samples. A, Immunized monkeys (nos. 5–8) received 100 µg of FimCH complex coformulated with MF59 adjuvant at study weeks 0, 4, and 48. Control monkeys (nos. 9–12) were inoculated with MF59 in PBS at same time points. Antibody responses to FimH were assessed by ELISA with FimH T3 as capture antigen. B, FimH IgG antibodies in vaginal wash samples obtained just before (week 47) and 2 weeks after (week 50) final immunization. Wash samples were diluted 1:2 in 0.5% bovine serum albumin (BSA), 0.5% milk, and 0.2% azide before analysis. Antibody levels were recorded as actual OD at 405 nm; values <2× background were considered negative. C, Functional inhibitory titers in serum samples measured by ability to block binding of type 1–piliated bacteria (Escherichia coli NU14) to transformed human bladder J82 cell line. Type 1–piliated NU14 were directly labeled with fluorescein isothiocyanate (FITC) and incubated with 1×10⁶ J82 bladder cells at a ratio of 250 bacteria/cell in presence of preimmune or hyperimmune serum (47- and 50-week samples) from monkeys and allowed to mix with bacteria for 30 min at 37°C. After multiple washes, samples were assayed by flow cytometry (FACStar PLUS; Becton Dickinson, San Jose, CA) as described elsewhere [5]. Mean channel fluorescence was used as indicator of FITC-labeled bacteria bound to J82 bladder cells. % inhibition for 47- and 50-week samples was determined relative to preimmune samples from each monkey. All serum samples were diluted 1:50 in PBS before analysis. D, Functional inhibitory antibodies in vaginal washes evaluated in E. coli trimannose binding assay. Immulon-4 plates (Dynex Technologies, Chantilly, VA) were coated with 2.5 µg/mL (100 µL/well) of trimannose-BSA (V-Labs, Covington, LA). Type 1–piliated NU14 (8.0 × 10⁷ cfu/mL) were added to each well, incubated at 37°C for 1 h, and, after extensive washing, bound bacteria were detected with 1:400 dilution of anti-E. coli–horseradish peroxidase–conjugated antibody (Biodesign, Kennebunk, ME). OD₄₅₀ readings of samples established full signal values (FSV) for binding to trimannose (~2.0). Additional samples were run in presence of 1:2 dilutions of vaginal wash samples to assess inhibition: % inhibition = [(FSV – sample value)/FSV] × 100. All samples were run in triplicate.

Samples from all immunized monkeys (figure 1B). Three of the 4 immunized monkeys had increases in FimH-specific IgG antibodies after the final booster immunization of 3- to 8-fold. One of the 4 immunized monkeys (monkey 5) had no increase in absolute vaginal IgG antibodies to FimH despite a >30-fold increase in serum antibody titers.

Prior studies in mice [5] showed a correlation between the ability of FimH antibodies to block binding of type 1–piliated bacteria to mannose or mannose-containing receptors and protection against in vivo infection. We tested serum and vaginal washes for the presence of inhibitory antibodies, using the 2 different assays described in figure 1. Although high levels of
inhibitory antibodies were observed in serum samples from all 4 immunized monkeys after a booster immunization at week 48 (figure 1C), only 3 of 4 vaccinated monkeys had an increase in overall FimH-specific IgG titers and functional inhibitory titers in the vaginal samples (figure 1D).

Protection from infection by type 1–piliated E. coli. All 8 monkeys were infected 18 days after the final immunization with a 1-mL inoculum containing 10^8 type 1–piliated NU14 bacteria. Urine samples were obtained on days 2, 4, 7, 12, and 14 after challenge to determine number of bacteria per milliliter of urine, as a measure of infection. Urine samples were also tested for leukocytes as an indicator of inflammation. Three of the 4 immunized monkeys were completely protected from bladder infection (table 1) and had no detectable bacteria or leukocytes in the urine on day 2 (limit of detection, 10^2 cfu/mL urine as in humans) and throughout the study. The immunized monkey that was not protected from initial colonization (monkey 5) was the only animal that failed to show an increase in FimH-specific and functional antibodies in vaginal secretions after booster immunization (figure 1D). All 4 control monkeys developed cystitis and had E. coli and leukocytes in their urine, which correlated with colonization (table 1).

Effect of adhesin vaccine on normal gut flora. No significant differences between immunized and control monkeys were observed throughout the study with regard to mean E. coli recovered from fecal suspensions from each group of monkeys. To examine whether the vaccine caused a shift in subpopulations within the coliform bacteria, biochemical characterization of the individual coliform isolates was done using the PhP assay. Samples were compared before and after vaccination (or placebo) in the 2 groups of monkeys. All monkeys in both vaccine and control groups had high Sp values when sequential samples were compared, indicating they carried similar coliform bacteria in their feces throughout the study. No significant differences in the populations were observed. Thus, systemic vaccination with the FimH adhesin does not appear to affect the normal gut flora.

Discussion

Our results demonstrate that a FimH vaccine mediates protection against cystitis in a primate model. The conclusions that can be drawn about differences or lack of differences between the groups was limited by the few animals studied. Nonetheless, our results clearly suggest that the FimH vaccine induced protective immunity. In natural infections, colonization of the vaginal mucosa precedes infection of the bladder [13], and adequate immune responses to the invading pathogen may be required in the vaginal mucosa as well as the bladder to provide maximal protection. We showed that IgG antibodies to FimH were also present in vaginal secretions, and the level of antibodies in secretions increased in concert with plasma IgG levels to FimH after booster immunizations. Functional antibody responses, as measured by their ability to block in vitro binding of type 1–piliated bacteria to isolated bladder cells or to mannose, were detected in both serum and vaginal wash samples. These results parallel observations in mouse studies [5].

Of interest, the vaccinated monkey that was not protected from bacterial colonization, monkey 5, was the only animal in the vaccine group that did not have an increase in IgG FimH antibody levels in vaginal secretions after the final boost before challenge with type 1–piliated E. coli. Although it is not clear why this monkey did not exhibit anti-FimH antibodies in secretions, the lack of protection is consistent with the notion that antibody levels in secretions may be more significant than overall serum antibody titers in predicting protective responses against mucosal infection [14]. For the 3 protected monkeys, our data clearly demonstrate that functional IgG antibodies against the FimH adhesin can block mucosal infections by uropathogenic E. coli, when such antibodies transudate into urogenital secretions.

The monkey studies reported here were done with a single cystitis isolate as a proof of principle. Studies in mice have demonstrated that the FimCH vaccine produced from a single strain of E. coli protects against a wide range of E. coli clinical isolates of different serotypes, including strains obtained from women with recurrent infections (unpublished data). These randomly selected E. coli strains have highly conserved FimH sequences and include strains that express the different type 1 pilus phenotypes [15]. In addition to type 1 pili, many of these strains are capable of expressing a variety of other pilus types, including P and S pili, which are associated with uropathogens [3].

Although this primate study strongly supports development of a FimH vaccine to block primary infections, it is important to note that a large percentage of women experience recurrent urinary tract infections after a primary episode. Future human studies, both in women with no previous history of infection and in women with ≥1 prior infection, should help determine whether this vaccine is effective in preventing recurrent infections as well as in blocking primary infections.

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References

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