Persistence of Escherichia coli Bacteriuria Is Not Determined by Bacterial Adherence

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The role of bacterial adherence in the persistence of bacteria in the human urinary tract was analyzed. Women with chronic symptomatic urinary tract infections were subjected to deliberate colonization with nonvirulent Escherichia coli, after eradication of their current infections. E. coli organisms were instilled into the bladder through a catheter. The strain used for colonization, E. coli 83972, was isolated from a patient with stable bacteriuria. It lacked expressed adherence factors and did not belong to the uropathogenic O:K:H serotypes. Strain 83972 was transformed with the pap and pil DNA sequences encoding Galα1-4Galβ- and manno-specific (type 1) adhesins. Patients were colonized with a mixture of the wild-type and the transformed strains. E. coli 83972 caused stable bacteriuria for >30 days in 7 of 12 individuals. In contrast, the Galα1-4Galβ-recognition or manno-binding transformants were eliminated within 48 h. The consistent superiority of the wild-type strain in establishing stable bacteriuria when compared with the adhesive transformants did not appear to be due to differences in growth rates or to plasmid segregation. Rather, the transformants expressing the adhesin determinants were selectively eliminated by the host. This suggested that the acquisition of adherence factors is not sufficient to increase the fitness of E. coli for survival in the urinary tract of humans.

Deliberate colonization of the human urinary tract with avirulent bacteria has been tested as an "ecological approach" to treatment of patients with urinary tract infection (UTI) that is refractory to other therapy (8). The choice of bacteria for such therapy is complicated, however, since the characteristics determining bacterial persistence in the urinary tract have not been identified.

Attachment enhances the virulence of Escherichia coli for the urinary tract (30, 31). Attachment has also been thought to promote bacterial persistence, by allowing E. coli to resist elimination in the urine flow during voiding (9, 22, 28). Adhesion is a consequence of the specific interaction between bacterial surface adhesins and carbohydrate receptors on host cells (16, 20). The bacterial chromosomal DNA sequences pap and pil (14, 21) encode fimbiae and adhesins which bind to the globo series of glycolipids (Galα1-4Galβ) on urogenital epithelial cells (16), or to manno residues on glycoproteins (6, 7, 33). Adhesins specific for both receptors enhanced bacterial persistence in the kidneys and bladders of mice and primates (9, 22, 25).

The hypothesis that adherence contributes to colonization of the human urinary tract is, however, contradicted by several observations. First, attachment to the globo series of glycolipids induces an inflammatory response, which facilitates bacterial clearance (5, 18, 19). Second, although adherence is a characteristic of strains isolated from patients with acute pyelonephritis, it is not typical of the strains which colonize patients with asymptomatic bacteriuria (ABU) for extended periods (24, 30). The role of adherence in bacterial persistence in the human urinary tract has, however, not been directly tested.

In this study we decided to pursue the ecological approach to the treatment of UTIs and to analyze how adhesion contributes to bacterial persistence in the human urinary tract.

MATERIALS AND METHODS

Patients. Women with a history of recurrent symptomatic UTIs refractory to conventional antibiotic therapy volunteered to participate. Their ages ranged from 26 to 73 years with a mean of 58 years. Characteristics of individual patients are listed in Table 1. Postvoid residual urine in the absence of bacteriuria was measured by catheter; three patients had significant retained volumes. The average serum creatinine was 85 mmol/liter (range, 58 to 109 mmol/liter). One patient had previously undergone a unsuccessful colonization attempt with lactobacilli as part of another study (8). The present study was approved by the Medical Faculty Ethics Committee, University of Göteborg, and informed consent was obtained from each patient. The Swedish Recombinant DNA Committee was notified.

Bacteria. Isogenic E. coli strains were prepared that differed in adherence properties. The wild-type E. coli isolate 83972, serotype ON:KN, carrying one plasmid of 1.2 kb, was used for colonization and as a recipient for transformation. It had been carried for 3 years by a girl with ABU, who showed no deterioration of renal function as tested by renal concentrating capacity. This strain was stored in deep agar cultures and maintained by passage on Trypticase soy agar (TSA; BBL, Cockeysville, Md.). E. coli 83972 was phenotypically negative for both manno- and Galα1-4Galβ-recognizing adhesins and did not attach to human uroepithelial cells in vitro. The strain was genotypically negative for pap-homologous DNA encoding pil and adhesins specific for the Galα1-4Galβ receptor but genotypically positive for the pil sequences encoding type 1 fimbiae (14). This strain

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was selected because of its documented ability to persist in the urinary tract.

The ABU strain, 83972, was subsequently subjected to transformation with the chromosomal DNA sequences *pap* and * pil*. The *E. coli* 83972 transformant Hu1061 received the plasmid pRHU845 with the *pap* DNA sequence inserted into the *EcoRI* site of the chloramphenicol gene of pACYC184 (14, 15). Hu1061 was resistant to tetracycline and was maintained on TSA containing 10 μg of this antibiotic per ml. The *E. coli* 83972 transformant Hu1053 received the plasmid pRHU960, with the *pil* DNA sequences inserted into the *Sall* site of the tetracycline gene of pBR322. The strain was resistant to tetracycline and was maintained on TSA containing this antibiotic. The 83972 transformant Hu1193 received pREG153 (a single-copy plasmid) without inserted DNA sequences. The strain was resistant to ampicillin and was maintained on TSA with 100 μg of ampicillin per ml.

**Inoculum mixture.** For colonization, bacteria were taken from the TSA plates and cultured overnight in tubes of antibiotic-free nutrient broth without glucose (23a). The bacteria were harvested by centrifugation and resuspended in 0.07 M phosphate-buffered saline (PBS; pH 7.2) at an estimated concentration of 10^8 bacteria per ml determined photometrically. The final inoculum was prepared by mixing equal parts of the strains. The concentrations were subsequently confirmed by measuring viable counts on antibiotic-free TSA or on TSA containing tetracycline or ampicillin.

Four mixtures were used for this study: (i) wild type (83972) alone; (ii) wild type (83972) mixed with the plasmid-bearing control Hu1193; (iii) wild type (83972) mixed with the adhering transformants Hu1053 and Hu1061; and (iv) a mixture of the two adhering transformants Hu1053 and Hu1061.

**Bacterial binding properties.** The expression of adhesins was analyzed by agglutination of erythrocytes and receptor-coated latex beads (4). Human erythrocyte blood groups AP, and Ap, and guinea pig erythrocytes were used as 3% (vol/vol) suspensions in PBS with or without the addition of α-D-methylmannoside. Bacterial suspensions of 10^9/ml were mixed with each of the erythrocyte suspensions on glass slides, and the agglutination was determined by the naked eye and designated as mannose resistant (MR), mannose sensitive (MS), or not present. The specificity of the MR binding for the GaLA1-4GaLB receptor was confirmed by using receptor-coated latex beads (Orion, Espoo, Finland).

Attachment to human uroepithelial cells was tested by using cells from the sediment of morning urine samples of one nonbacteriuric donor (27). Attachment to 20 squamous and 20 transitional epithelial cells was assessed by interference contrast microscopy, and adhesion was given as the mean number of bacteria attached to those cells.

**Bacterial growth rates.** The inoculum was prepared as described for colonization. For assessment of growth rates, the inoculum was diluted 1/100 in sterile PBS and 0.1 ml was added to 5 ml of nutrient broth, urine, or equal parts of urine and broth and cultured at 37°C. The urine was selected from successfully colonized patients. The optical density of the broth cultures was recorded, and viable counts were performed every hour. Differences in growth rate were evaluated by using the mathematical model of Crow and Kimura (3). The growth rate was estimated from the equations V = 1/ln(N/N₀) and V = 1/ln(DO/D₀), where N₀ and N are the live-cell densities and OD, and OD₀ are optical densities at two different times, 0 and t.

**Urinary antibodies.** The urinary antibody activity of the patients was measured by using the enzyme-linked immunosorbent assay (ELISA) (26). Microtiter plates (Nunc, Copenhagen, Denmark) were coated with heat extracts of *E. coli* 83972, 1053, and 1061. The heat extracts contained lipopolysaccharides and heat-stable proteins including lipfimbriae (23). Urine samples diluted in PBS-Tween were added in duplicate in 10-fold dilutions, starting from 1/2. The bound antibody was detected by using alkaline phosphatase-conjugated goat anti-human IgG, IgM, and IgA (Orion, Helsinki, Finland). The antibody activity is given as the density at 405 nm after a 10 min incubation with the substrate.

**Study protocol.** Prior to colonization, the patients were treated with oral antibiotics to eliminate preexisting bacteruria. Three patients received norfloxacin, two received cotrimoxazole, one received cefadroxil, and two did not require any antibiotics. To reduce the likelihood of reinfection between cessation of precolonization antibiotic treatment and inoculation with the study strains, we introduced the strains on three consecutive days starting 5 days after the last dose of antibiotic.

The patients were hospitalized for colonization and 24 h of observation; this was followed by outpatient follow-up. The colonization procedure was as follows. A postvoid residual urine sample was collected by catheter and cultured to confirm the sterility of the precolonization urine. After complete evacuation of the bladder, 10 ml of the bacterial mixture was introduced and the catheter was removed. During the subsequent 24 h, all urine specimens were microscopically analyzed for leukocytes and cultured. Daily urine samples were obtained for the next week, and weekly
PERSISTENT BACTERIURIA WITHOUT ADHERENCE

TABLE 2. Binding properties of E. coli in the inoculum mixture

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Hemagglutinationa</th>
<th>Attachmentb (bacteria/ cell)</th>
<th>Galá1-4Galß-treated latex beadsc</th>
</tr>
</thead>
<tbody>
<tr>
<td>83972</td>
<td>pap pil*</td>
<td>A1P1 - - -</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Hu1193</td>
<td>pap pil</td>
<td>A1P - - -</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Hu1051</td>
<td>pil*</td>
<td>MS MS MS</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Hu1061</td>
<td>pap*</td>
<td>MR - - -</td>
<td>+</td>
<td>+-</td>
</tr>
</tbody>
</table>

- A1P1 and A1P are human erythrocytes of these blood groups; GP are guinea pig erythrocytes.
- Attachment to uroepithelial cells from a donor of A1P, secretory blood group.
- Strength of agglutination.

TABLE 3. Bacterial persistence and host response in the colonized patients

<table>
<thead>
<tr>
<th>Patienta</th>
<th>Persistence (days)</th>
<th>WBC (10⁶/liter)b</th>
<th>CRP (µg/ml)c</th>
<th>ESR (mm/h)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>83972</td>
<td>Hu1053 Hu1061</td>
<td>1-1</td>
<td>90 1</td>
<td>7.3 0 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-2</td>
<td>226 - 9</td>
<td>11.9 0 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-1</td>
<td>18 1 1</td>
<td>7.2 0 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-2</td>
<td>232</td>
<td>6.8 0 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-3</td>
<td>176 &lt;1 1</td>
<td>8.9 0 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>100 1 2</td>
<td>9.6 0 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4f</td>
<td>1 1 1</td>
<td>5.0 0 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-1f</td>
<td>&lt;1 &lt;1 &lt;1</td>
<td>6.4 0 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-2</td>
<td>142 1 &lt;1</td>
<td>7.1 0 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-1f</td>
<td>2 1</td>
<td>5.5 0 48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-2</td>
<td>2 &lt;1 1</td>
<td>5.6 0 48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>9 1 2</td>
<td>7.3 0 26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8-1f</td>
<td>2 1</td>
<td>7.2 0 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8-2</td>
<td>59 1 1</td>
<td>5.7 0 8</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>88</td>
<td>1.8 2</td>
<td>7.4 0 18</td>
</tr>
</tbody>
</table>

- 1-1 indicates the first colonization attempt in patient 1; 1-2 indicates the second attempt, etc. Each colonization attempt involved up to three bacterial inoculations.
- WBC, white blood cells in serum. These numbers represent the highest value during colonization.
- CRP, C-reactive protein.
- ESR, erythrocyte sedimentation rate. These numbers represent the highest value during colonization.
- Patient 2 was also colonized with a mixture of the wild-type strain 83972 and the plasmid-bearing transformant Hu1193. Both strains persisted for more than 6 days.
- Inhibitory antibiotic present in urine.
- Fewer than 1 in 10⁶ revertants were found on day 1.
- Treated for cystitis symptoms.

RESULTS

E. coli strains. The wild-type strain 83972 and the plasmid-containing control strain Hu1193 were phenotypically negative for adhesins reacting with human or guinea pig erythrocytes and did not attach to human uroepithelial cells in vitro (Table 2). The transformant Hu1061 expressed adhesins inducing MR agglutination of human API erythrocytes and Galá1-4Galß-coupled latex beads and attached weakly to human uroepithelial cells (pil*). Hu1053 induced MS agglutination of guinea pig erythrocytes and attached to human uroepithelial cells, but did not agglutinate the Galá1-4Galß-treated latex beads (pil*).

Outcome of colonization. There were 15 colonization attempts in eight patients (Table 3). A mixture of strain 83972 and the two adhering transformants Hu1061 and Hu1053 was given on 11 occasions. A mixture of the two transformants Hu1061 and Hu1053 was given on two occasions (patients 6-1 and 8-1); a mixture of strain 83972 and the plasmid-bearing control Hu1193 was given on one occasion (patient 2-4), and strain 83972 alone was given on one occasion (patient 2-2). Patients 4 and 5 had bactericidal levels of antibiotic in their urine at the time of colonization and did not retain the inoculum.

Seven colonization attempts were successful, as defined by the presence of ABU for at least 30 days (average, 146 days). Failure of the first colonization attempt did not preclude successful subsequent inoculations in patients 2 and 8. Of three patients with long-term colonization who eventually lost the strain, one developed ABU with a new wild-type E. coli strain and two became abacteriuric.

The patients did not develop fever or any other signs of systemic illness, and only one patient (patient 7) described lower urinary tract symptoms. She complained of frequency and dysuria and required antibiotic therapy on day 9 of colonization. At the time of antibiotic treatment, only the ABU strain 83972 was cultured from her urine. Colonizations were not associated with significant elevations of leukocyte counts, C-reactive protein, or erythrocyte sedimentation rate. Patient 6 had an elevated erythrocyte sedimentation rate of 48 mm/h prior to colonization for unknown reasons, and this elevation remained unchanged during the colonization. Renal function as measured by serum creatinine was normal for all patients prior to colonization and did not deteriorate during the study. Bacteriuria was associated with pyuria in all cases, and the urinary leukocytes persisted for as long as the patients remained bacteriuric.

Persistence of the ABU strain and elimination of the transformant. The wild-type ABU strain successfully colonized the urinary tract in 7 of 12 patients (Fig. 1). The adhering transformants were typically eliminated within 2 days of...
inoculation. Their period of persistence is specified in Table 3 for each patient and colonization attempt. Figure 2 illustrates the persistence of the wild type and elimination of the adhering transformants in one patient.

All the successfully colonized patients retained the ABU strain, which established a population in the range of $10^7$ to $10^9$ CFU/ml of urine. The time limits given in Table 3 represent the time of follow-up rather than the time at which the bacteriuria was eliminated.

The possible mechanisms explaining the consistent elimination of the adhering transformants in the face of persistence of the ABU strain 83972 were investigated. These are discussed below. (i) The first is disadvantage afforded by plasmid carriage. A possible explanation for this involves differences in growth rate. The carriage of the recombinant plasmids and/or the expression of the adhesins may reduce the growth rate of the transformants. To assess the possible differences in growth rates, the ABU strain (83972) and the adhering transformants Hu1053 and Hu1061 were cultured in vitro in nutrient broth, urine from the patients, or mixtures of urine and broth. The growth of nonaerated cultures was monitored by measuring the OD$_{597}$. Figure 3 shows the results based on 10 experiments. The ABU strain grew faster than the transformants in urine and the broth-urine mixture, but the difference reached statistical significance only when strain 83972 was compared with Hu1061 (pap$^+$) in the broth-urine mixture. The time, $t$, required for these growth rate differences to result in exclusion of the transformants can be estimated by the following formula:

$$ t = \frac{1}{s} \ln \left( \frac{f_a(1 - i_d)}{i_d(1 - f_b)} \right) $$

where $s$ is the difference in growth rates and $f$ is the final and $i$ the initial frequency of the strains $a$ and $b$. Since each strain accounted for one-third of the inoculum given to the patients, $i$ is 0.333. If a final frequency for the wild-type strain is assumed to be 0.999 and that of the transformant strains to be 0.001, the time required for strain 83972 to become the exclusive resident in the broth-urine mixture was calculated to be 141 h. The transformant strains were actually eliminated in most cases within 48 h. Furthermore, the plasmid-bearing control Hu1193 did not differ in vitro growth rate and persisted for the same time. Taken together, this suggested that a factor(s) other than differential growth rates accounted for the elimination of the transformants.

There are also other disadvantages due to plasmid carriage. Patient 2 was inoculated with a mixture of the ABU

\[ \text{FIG. 1. Long-term persistence of the wild-type strain in the seven successfully colonized patients.} \]

\[ \text{FIG. 2. Persistence of the wild-type \textit{E. coli} strain and elimination of the adhering transformants. ABU, \textit{E. coli} 83972; MR, Hu1061; MS, Hu1053.} \]
strain (83972) and the plasmid-bearing, nonadhering control Hu1193. The adhering transformants were eliminated within 48 h. The plasmid-bearing control was retained for the 6 days that the follow-up lasted. The population size was calculated as an average of the viable counts in the urine samples collected on each day. They were (for strains 83972 and Hu1193) $0.3 \times 10^5$ and $3.8 \times 10^6$, respectively, on day 1; $2.2 \times 10^5$ and $5.6 \times 10^7$, respectively, on day 2; $3.2 \times 10^8$ and $2.1 \times 10^9$, respectively, on day 3; and $1.0 \times 10^9$ and $2.0 \times 10^9$, respectively, on day 6.

(ii) The second possible mechanism is segregation. The possibility that the transformants lost the recombinant plasmid and reverted to the wild-type strain was analyzed. The growth of each transformant in a mixture of equal parts of urine and the broth medium was determined. Emergence of the wild-type strain was not observed after 24 h for any at the transformants. The frequency of revertants was $< 1$ in $10^6$. Similarly, initial inoculation of patients 6 and 8 with a mixture consisting only of the two transformants did not result in the emergence of the wild-type strain ($< 1$ in $10^6$).

(iii) The third possible mechanism is production of inhibitors. The ability of the ABU strain to inhibit the growth of the transformants was assessed by coculture experiments. Differences in growth rates were not observed when the transformants were cultured separately or with the wild-type strain. Furthermore, the cross-streaking technique did not provide evidence of growth inhibition.

Growth rates in urine of colonized and noncolonized patients. The difference in acceptance of the ABU strain by patients could be due to individual variation in the ability of urine to support bacterial growth. In vitro growth of the wild-type strain in urine from successfully and unsuccessfully colonized patients was therefore compared. Again, significant differences in growth rate were not found (data not shown).

Urinary antibody activity. The difference in acceptance of the strain could also be due to the level of preexisting immunity. The urinary antibody activity of the patients is shown in Table 4. The antibody activity was low. There was no significant difference in the antibody activity of the

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TABLE 4. Urinary IGA antibody activity

<table>
<thead>
<tr>
<th>Outcome of colonization</th>
<th>Patient</th>
<th>Day of sample</th>
<th>Antibody activity $(A_{405}, 100$ min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Success</td>
<td>1-1</td>
<td>5</td>
<td>121</td>
</tr>
<tr>
<td>Failure</td>
<td>1-2</td>
<td>4</td>
<td>29</td>
</tr>
<tr>
<td>Success</td>
<td>2-1</td>
<td>14</td>
<td>114</td>
</tr>
<tr>
<td>Success</td>
<td>2-2</td>
<td>6</td>
<td>56</td>
</tr>
<tr>
<td>Success</td>
<td>2-3</td>
<td>2</td>
<td>120</td>
</tr>
<tr>
<td>Success</td>
<td>3</td>
<td>6</td>
<td>44</td>
</tr>
<tr>
<td>Failure</td>
<td>5-1</td>
<td>10</td>
<td>67</td>
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<td>Success</td>
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<td>3</td>
<td>23</td>
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<td>6-1</td>
<td>3</td>
<td>61</td>
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<td>Failure</td>
<td>8-1</td>
<td>2</td>
<td>$&lt; 10$</td>
</tr>
<tr>
<td>Success</td>
<td>8-2</td>
<td>4</td>
<td>$&lt; 10$</td>
</tr>
</tbody>
</table>

* An explanation of the numbers is given in Table 3, footnote a.

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DISCUSSION

Stable bacteriuria was created by deliberate instillation of *E. coli* into the urinary bladders of women. The patients were given a mixture of a wild-type strain isolated from a patient with long-term ABU and two isogenic adhering strains. The isogenic strains were obtained by transformation of the ABU strain with DNA sequences encoding Galα1-4Galβ and mannose-sensitive adhesins. The expression of adhesins by the transformed strains was expected to enhance their persistence. They were, however, both promptly eliminated from the urinary tract. In contrast, a transformant containing a plasmid vector without inserted adherence DNA sequences was retained. Thus, adherence did not promote bacterial colonization of the urinary tract in the patients studied.

Several mechanisms could explain this rapid elimination of the transformants. The adherent phenotype of the transformants appeared to be responsible for their selective elimination. This was illustrated clearly by patient 2, who on separate colonizations retained the ABU strain or the plasmid-bearing control, but was never able to retain the two adhering transformants. We have recently shown that the attachment of *E. coli* to Galα1-4Galβ-containing receptors initiates an inflammatory response which leads to bacterial elimination by the host (5, 18). Animals lacking this response, either owing to genetic defects or secondary to treatment with anti-inflammatory agents, become chronically infected (19, 29). In the present study, the colonized patients showed signs of a local inflammatory response such as pyuria and urinary interleukin-6 excretion (13). In contrast, there was no significant change in the systemic host response parameters, i.e., temperature, C-reactive protein, erythrocyte sedimentation rate, and blood leukocyte counts. The mucosal response of the colonized hosts may therefore have been the basis for the selective elimination of adhering transformants. Bacterial adhesins may even facilitate the phagocytic elimination of the strains (29).

The patients in this study all had multiple prior episodes of UTI. Reiterated mucosal exposure to bacterial antigens is known to elicit a local immune response, but such responses often are of short duration. On the other hand, preexisting
local immunity might explain the selective elimination of the adhering strains. We therefore analyzed the urinary antibody activity at the time of colonization. The urinary antibody activity against the colonization strains as well as against a pool of heat-stable E. coli antigens was low in all patients and was not significantly different between the successfully colonized patients and the colonization failures.

The adhering transformants were expected to grow more slowly than the wild-type strain, as a function of the recombinant plasmid. The differences in growth rate were, however, not significant. Indeed, the disadvantage in growth rate of the transformants was not of sufficient magnitude to explain their elimination. The ability to support bacterial growth did not vary as extensively as expected among the urine samples from the patients and therefore did not appear to account for the different outcomes of colonization. The dominance of the wild-type strain may also have been due to segregation of the recombinant plasmid. Evidence against segregation of the plasmid was, however, provided in vivo in two patients who received only the transformants; they eliminated the strains as rapidly as the other patients, and the emergence of the wild-type phenotype was not observed. Emergence of the wild-type strain was also not seen during in vitro culture of the transformants. Elimination of the adhering transformants might be due to their adherence to the urinary tract mucosa. This was considered unlikely. The patients were monitored with repeated cultures for several months. Occasional excretion of the adhering transformants was not observed. The possible change in bacterial characteristics in response to in vivo conditions was analyzed from the adhesin expression by the bacteria recovered from the patient urine. The wild-type strain E. coli 83972 did not express type 1 fimbriae under these conditions or after in vitro culture in static Luria broth. In contrast, the colonies recovered from the tetracycline agar expressed MR adhesins, whereas those recovered from the ampicillin agar expressed MS adhesins.

The efficiency with which the inflammatory response eliminates bacteria from the urinary tract depends on bacterial properties in addition to adherence. Bacteria which have complete O antigens and capsule are more resistant to elimination by inflammation than are rough strains (29). Strain 83972 lacked definable O and K antigens. The present study suggested that the acquisition of adhesive properties is detrimental for a strain lacking surface components which protect against the host inflammatory response. Adherence may well contribute positively to the persistence and virulence in the urinary tract of a strain which, in contrast to the one used here, was equipped with a complete set of such properties.

These observations raise the question of why uropathogenic E. coli organisms maintain DNA sequences encoding adhesins (17). The pil sequences are found in virtually all E. coli strains regardless of origin (24). The main proposed function of the MS adhesins is to promote colonization of the large intestine, which is the natural ecologic niche for E. coli and other enterobacteria (6, 34). Type 1 fimbriae were recently proposed to also enhance the colonization of the oral cavity (1). pap DNA sequences are characteristic of uropathogenic E. coli. They occur in about 81% of strains associated with pyelonephritis, 65% of strains associated with cystitis, and 56% of strains associated with ABU, but in only 16% of the normal fecal E. coli strains (24). Receptors for the Galα1-4Galβ-specific adhesins occur both in the large intestine and in the urinary tract (16, 34). This specificity may therefore contribute to the large intestinal colonization preceding UTI, as well as to the virulence in the urinary tract. The results of the present study suggest that the acquisition of the P or type 1 fimbrial phenotype is not sufficient to enhance the fitness of bacteria for the urinary tract.

The growth of one bacterial strain in the urinary tract has been proposed to protect against invasion by other, possibly more virulent bacteria (8, 10–12, 32). Thus, girls and women with untreated ABU developed symptomatic UTI less frequently than those receiving antibiotics (10–12). The patients in our study had not responded to or not tolerated conventional antibiotic therapy. The rate of successful colonization of 58% (7 of 12 cases) was therefore encouraging. With the exception of patient 7, the strains did not cause symptoms or objective evidence of systemic illness. The successfully colonized patients experienced subjective clinical improvement. Normal renal function was maintained in all cases. This is in concurrence with the findings of Tencer (32) and Hansson et al. (12). Tencer described 40 women with untreated ABU who were monitored for 15 years without evidence of renal deterioration, and Hansson et al. reported similar findings in girls with untreated ABU. The ability of the wild-type ABU strain to colonize different hosts makes it a candidate for future use in ecological therapy against recrudescent cases of UTI.

The results of the present study illustrate the problems associated with the virulence concept. The ABU strain (83972) was able to establish and maintain a bacterial population around 10⁶/ml in the urine of several individuals, to activate a mucosal inflammatory response, but not to cause systemic disease. This limited “virulence” may be due to successful adaptation to the long-term persistence in the favorable ecological habitat of the urinary tract. The results also illustrate our limited understanding of the properties which determine bacterial persistence in the urinary tract and at other mucosal sites.

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REFERENCES


7. Firon, N., I. Ofek, and N. Sharon. 1983. Carbohydrate speci-
PERSISTENT BACTERIURIA WITHOUT ADHERENCE


