Experimental *Escherichia coli* Ascending Pyelonephritis in Rats: Changes in Bacterial Properties and the Immune Response to Surface Antigens

I. MATTISBY-BALTZER,1,2* L. Å. HANSON,1 B. KAUSER,2 P. LARSSON,3 S. OLLING,3 AND C. SVANBORG-EDÉN

Departments of Clinical Immunology,1 and Clinical Bacteriology,2 Institute of Medical Microbiology, University of Göteborg, and Department of Clinical Pathology, Östra Sjukhuset,3 Göteborg, Sweden

Received 27 August 1981/ Accepted 15 October 1981

Systemic and urinary antibody responses were examined in rats with experimental ascending pyelonephritis caused by *Escherichia coli* O6K13H1. During 12-month follow-up of the infections, bacterial characteristics of the urinary and renal isolates were followed: O and K antigenicity, sensitivity to the bactericidal effect of normal human serum, capacity to attach to urinary tract epithelial cells, hemolytic activity, biochemical pattern, and virulence. During the long-term infection, the urinary and renal bacterial isolates changed in O and K antigenicity, serum sensitivity, and virulence. The adhesive capacity of the bacterial isolates did not change, possibly explaining the persistence of the bacteria in the urinary tract. The serum anti-O6 antibody levels remained high during the entire 1-year observation period, especially in the rats with renal involvement. Urinary anti-O6 antibodies were also found. The serum and urinary antibodies could have played a role in bringing about the observed changes in bacterial characteristics. Antibodies to lipid A were recorded in 9 of 16 rats with pyelonephritis and renal scarring and in 1 of 9 rats not having pyelonephritis or renal bacterial growth.

*Escherichia coli* bacteria causing symptomatic urinary tract infections (UTI) in humans possess several virulence factors. *E. coli* isolated from patients with such different forms of UTI as nonobstructive acute pyelonephritis, acute cystitis, and "asymptomatic" bacteriuria (ABU) seem to differ with respect to O and K antigens, O antigenicity, sensitivity to bactericidal activity, and adhesiveness to human urinary epithelial cells (6, 14–16, 28, 31). Bacteria isolated from the urine of patients with ABU are more often spontaneously agglutinating (SA), less adhesive, and more serum sensitive (15, 31) than bacteria isolated from patients with cystitis or pyelonephritis. *E. coli* bacteria sequentially isolated from untreated ABU patients have also shown consecutive changes, with the bacteria becoming more sensitive to the bactericidal action of serum and becoming SA (15). These changes may be induced by the antibodies to the O and K antigens of the infecting organisms, which appear in the serum and urine of the patients (27).

A similar "antigenic drift" has been reported for other microorganisms exposed to antibodies (1, 11, 20, 23).

The aim of this investigation was to study different characteristics of the infecting microorganisms during an experimentally induced long-term infection of rat urinary tract, trying to reveal bacterial changes relating to time and the antibody response to the O and K antigens appearing in the animals. In addition, the antibody response to lipid A in serum and urine was studied in relation to the appearance of renal scarring in the infected rats. High titers of antibodies to lipid A have been recorded in patients with upper UTI and renal scarring of recent origin (17).

**MATERIALS AND METHODS**

**Experimental long-term ascending pyelonephritis.** (i) **Bacterial strain.** *E. coli* O6K13H1 (World Health Organization designation Su 4344/41) was used for infection. This strain was homogeneous and stable as checked by serotyping and testing of serum sensitivity of 10 colonies picked from a plate with *E. coli* O6K13H1. Earlier infection experiments with this strain also revealed its stability. The bacteria were cultured overnight and washed with phosphate-buffered saline, and the concentration was adjusted to 2 × 10⁸ to 4 × 10⁸/ml.

(ii) **Procedure.** Female Sprague-Dawley rats weighing about 200 g were injected intravesically with 0.6 ml of *E. coli* O6K13H1 under slight ether anesthesia (10). Forty animals were infected, and 10 were injected with nutrient broth as controls. The kidneys were inspected 1 week after infection by laparotomy for diagnosis of pyelonephritis. The animals were killed at 1, 2, 3, or 4 months, or 1 year after the infection. Serum and urine samples were collected during the entire observation period.
period. The control rats were kept in separate cages to prevent them from being colonized by the bacteria used for infection.

(iii) Confirmation of urinary tract infection. The kidneys were removed under aseptic conditions for microscopic and macroscopic examination, and grading of the lesions was performed as earlier described (10): healthy indicates no inflammatory foci on microscopic examination of the kidneys; pyelitis indicates inflammatory foci in the renal pelvis, which were not observed in the parenchyma; and pyelonephritis indicates lesions from microscopic inflammatory foci to macroscopic confluent abscesses. The urinary bladders were also removed for microscopic examination. Bacteria were cultivated by pressing the middle part of the sectioned kidney or the opened urinary bladder against agar plates.

Characterization of isolated bacteria. (i) Serotyping. The E. coli strains isolated from the kidney, urinary bladder, or urine were O and K serotyped (7, 13), with "06" indicating weak O6 agglutination. All specimens were cultured on Drigalski plates and one colony was taken for typing. Colonies on the same plate which differed in appearance were all typed. The bacterial strains were stored as deep agar stab cultures.

(ii) Biochemical analysis. Biochemical characterization of isolated strains was performed with a test kit consisting of 20 tests (API 20E, Analytab Products, Plainview, N.Y.). Bacterial strains considered to be derived from the original E. coli O6K13H1 had the same biochemical pattern as the original strain.

(iii) Hemolysis. The hemolytic capacity of the isolated strains was tested on nutrient agar plates with 5% washed horse erythrocytes. E. coli O6K13H1 used for infection was hemolytic.

(iv) Serum bactericidal sensitivity. The bacterial isolates were tested for sensitivity to serum bactericidal activity (22). This test was performed with normal human serum. E. coli O6K13H1 was resistant to bactericidal activity.

(v) Attachment of E. coli to rat uroepithelial cells. Our in vitro model was used (30), with uroepithelial cells from the fresh pooled urinary bladder of 20 healthy rats (E. coli O6K13H1; mean, 70 bacteria per epithelial cell; standard error, 10 bacteria per cell). The chi-square test was used for statistical evaluation.

(vi) Virulence of isolated bacteria. Strains isolated from the urine of rats with long-term infections were tested for capacity to reinfect new rats. This experiment was repeated at least twice for each bacterial strain.

Antibody determination. The enzyme-linked immunosorbent assay of Engvall and Perlmann (2) was used with some modification (18). Anti-rat immunoglobulin G (IgG), IgM, and IgA were purchased from Nordic Laboratories (Tilburg, The Netherlands), and alkaline phosphatase was from Sigma Chemical Co. (St. Louis, Mo.). E. coli O6 lipopolysaccharide (LPS) was prepared by the phenol-water procedure (34), and K13 was purified from E. coli O22K13H1 (7). The phenol-chloroform-petroleum ether method (3) was used for preparation of LPS from a rough mutant termed E. coli EH 100 (4). Lipid A was isolated from this LPS (18). The concentrations of O6 LPS, K13, and lipid A for coating of the tubes were 0.01, 0.05, and 0.005 g/liter, respectively. Serum and urine were diluted in 10-fold steps, with starting dilutions of 1:100 for serum and undiluted to 1:5 for urine. Urines collected at various times from the same rat were pooled in some instances.

The enzyme-linked immunosorbent assay titer was defined as the log_{10} of the sample dilution, giving an extinction value at 405 nm of 0.125 above the background reading. One or two reference sera were included in each run. Two sera tested against O6 antigen 7 and 24 times showed a standard deviation of ±0.20 log_{10} unit for the IgG and IgM conjugates.

Statistics. The Wilcoxon test for two samples, the chi-square test, and the Kolmogorov-Smirnov test were used (24).

RESULTS
Occurrence of pyelonephritis and persistence of bacteria. Forty rats were infected with E. coli O6K13H1. Nine rats died early either at the time of the laparotomy or from the infection. One rat was excluded because gram-negative bacteria other than E. coli were isolated from the kidneys. The frequency of pyelonephritis was 60% (17 of 30).

Thirteen of 18 animals killed 1 to 4 months after infection had pyelonephritis or pyelitis or both (Table 1). Bacteria were present in the kidneys of all but 2 of these 13 rats. None of the five animals still healthy (i.e., no pathological changes of the kidneys), after the infection had bacteria present in their kidneys. At 12 months, all seven rats with pyelonephritis and one of two rats with only pyelitis had bacterial growth in their kidneys. None of the three healthy rats had renal bacterial growth.

Seven of the 10 control rats injected intravesically with nutrient broth and killed at various times had histologically normal kidneys, although 1 of these 7 had bacterial growth of a spontaneously agglutinating strain with non-typeable K antigen. One of the three rats with affected kidneys died spontaneously. The other two had no gram-negative bacteria present in their kidneys. One of these two rats developed interstitial nephritis which was not seen in any other rat. The other one showed pyelonephritic changes.

<p>| TABLE 1. Persistence of E. coli bacteria in rat kidneys after intravesical injection of E. coli O6K13H1 |</p>
<table>
<thead>
<tr>
<th>Grade of infection</th>
<th>Time (mo) after bacterial injection</th>
<th>No. of animals</th>
<th>No. with bacterial growth in kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyelonephritis</td>
<td>1–4</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Pyelitis</td>
<td>1–4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Healthy</td>
<td>1–4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>12</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Pyelitis</td>
<td>12</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Healthy</td>
<td>12</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>
Characterization of isolated bacteria. (i) Serotyping. The serotypes of bacteria isolated from the urine of rats with or without renal bacterial growth are shown in Fig. 1a and b. E. coli O6K13 ("06"K13 included)-positive bacteria in the group of rats with no renal bacterial growth (11 rats) were less than 40% during the observation period of 4 months (Fig. 1b). E. coli O6K13 was isolated from the urine in 6 of these 11 rats on at least one occasion. In contrast, 80% or more of the urinary bacterial isolates from the rats with renal bacterial growth were O6 ("06") or K13 or both (Fig. 1a). Bacterial strains typed as O6:(not K13) and (not O6):K13 were only found among these rats.

The distribution of O6K13 among the renal bacterial isolates was about 50%, whereas the remaining isolates consisted of "06"- or K13-positive (or both) or SA strains. Table 2 shows the serotypes of the bacteria sequentially isolated from the urine of eight rats followed for 8 months after the onset of infection. It also shows the bacteria isolated from the kidneys of those rats taken at 12 months after the infection. At 5 and 8 months, all urinary bacterial isolates were "06":K13, "06" but K13 negative, or SA. Of the renal isolates taken from the right kidney, four showed O6K13 reactivity, as did six of the eight isolates from the left kidney.

Urinary bladders from 22 rats were also cultured. No bacterial growth could be recorded when the corresponding kidneys were sterile. The serotypes isolated from the urinary bladder were also consistently found in one or both of the corresponding kidneys.

The O6 antigen was a somewhat labile characteristic of the bacterial strains isolated from the infected rats compared with the original E. coli O6K13H1 strain. This was measured by retesting 30 O6- and K13-positive strains ("06" included). In contrast, K13 antigenicity was a stable feature. The original E. coli O6K13H1 was always positive for O6 and K13 when tested. The analysis showed that the strains isolated from the infected rats were different from the original E. coli in their O antigen.

(ii) Biochemical analysis, hemolysis, serum bactericidal sensitivity, and attachment to rat uroepithelial cells. A total of 43 bacterial strains sequentially isolated from the urine of seven infected rats were analyzed. Ninety-seven percent of the tested bacterial strains with serotype O6K13, "06":K13, or negative for one of the antigens had all four characteristics (36 of 38) or three of them (1 of 38) in common with the original E. coli O6K13H1 (Table 3). One strain, which was only positive for O6, showed no other characteristics in common with the original E. coli strain and was not considered as being derived from it. Two SA strains, of which one was K13 positive and the other was not, differed from the original E. coli in serum sensitivity. Four strains negative for both antigens only had one parameter (serum bactericidal resistance) in common with E. coli O6K13.

Sequentially collected strains from 5 of the 14 rats (36%) which had bacterial growth in their kidneys and had been followed for at least 3 months after infection showed increased serum bactericidal sensitivity, whereas those from the remaining 9 rats did not (Fig. 2). This increased sensitivity of the bacterial strains isolated from the urine was also seen in the bacteria isolated from one or both of the kidneys, except in one case.

No serum-resistant strains were found among the seven tested SA bacterial isolates.

All bacterial isolates of serotypes O6K13, O6
TABLE 2. O:K antigen distribution among eight rats with renal bacterial growth followed for 8 monthsa

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Urinary isolates at time (mo) after infection:</th>
<th>Renal isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.75</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>33</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>34</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The renal isolates were taken at 12 months. + +, O6:K13 positive; − −, (not O6):(not K13); (+) +, weakly agglutinating with anti-O6 serum. ND, Not determined.

only, or K13 or SA attached to rat uroepithelial cells to about the same degree as did the original E. coli O6K13. The strains which showed a significantly lower degree of attachment were negative for both O6 and K13 and were not SA.

(iii) Virulence. A statistically significant lower capacity to induce pyelonephritis was observed for one strain isolated at 5 months compared with the control (Table 4). The other four bacterial isolates from different rats or samples collected earlier from the same rat did not differ in virulence compared with the control.

Antibody response to O6, lipid A, and K13. (i) Antibody response to O6 LPS. The infected rats were divided into three groups. Eight rats with no bacterial growth and no pathological changes of the kidneys were referred to as healthy. The second group consisted of 17 rats with pyelonephritis and bacteria present in their kidneys. Five animals with pyelitis or pyelonephritis, but without gram-negative bacteria in their kidneys, were not included in the two former groups, since the presence of gram-positive bacteria could not be excluded.

Infected rats with pyelonephritis and renal bacterial growth showed higher serum antibody titers of all three tested immunoglobulin classes, compared with rats with healthy kidneys and no growth (Fig. 3). No obvious relationship was seen between the antibody response in rats with pyelonephritis and the various antigenic patterns of the urinary bacterial isolates during the observation period (Fig. 1).

During the first month after bacterial injection, urinary IgA antibodies to O6 LPS were only found in rats with pyelonephritis (5 of 15). Three to 6 months after the infection, both IgG and IgA antibodies were recorded in the pyelonephritis group (5 of 10 and 7 of 10, respectively). Six of seven analyzed rats with renal bacterial growth which were followed for 1 year (Table 2) had both IgG and IgA urinary antibodies. Antibodies of the IgG and IgA classes were also found in the healthy animals between 8 and 12 months after injection (2 of 2).

Two of the six noninfected control rats developed serum anti-O6 antibodies of rather low levels. These two rats showed IgM antibodies 2 weeks after the injection of nutrient broth (1 week after the laparotomy).

(ii) Antibody response to lipid A. In the pyelonephritis group, 6 of 16 rats had a serum antibody response to lipid A within the first 4 months of infection, and 4 of 8 had one after 8 months of infection (Table 5). Four of the total of nine rats with lipid A antibodies were those

TABLE 3. Biochemical characteristics, hemolysis capacity, serum bactericidal sensitivity, and attachment to rat uroepithelial cells of 43 bacterial strains sequentially isolated from the urine of seven infected rats

<table>
<thead>
<tr>
<th>No. of parameters in common with original E. coli O6K13</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>O6:K13</td>
<td>O6:(not K13)</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

a These two strains were SA and sensitive to serum bactericidal activity; the original E. coli O6K13 strain was not.
with the most extensive pyelonephritic changes. One of these four rats, which showed definite progression in its renal damage, was also the one with the highest IgG antibody titer recorded 8 months after the infection. The other five rats with anti-lipid A antibody activity could not be separated from the remaining rats with pyelonephritis, judging from their degree of renal involvement. No relation could be established between the occurrence of anti-lipid A antibodies and anti-O6 antibodies. Serum antibodies to lipid A were not observed in the nine healthy rats until 12 months after infection, when one rat had high levels of IgG and IgM antibodies.

(iii) Antibody response to K13. Only IgM serum antibodies to K13 were found (nine rats analyzed). There was no difference in the antibody response between healthy rats and rats with pyelonephritis.

DISCUSSION
We studied bacterial strains isolated from the kidneys and from sequentially collected urine of
rats with persistent UTI induced by urinary bladder injection of *E. coli* O6K13H1. Excluding the bacterial isolates, which were negative for both O6 and K13, most were probably derived from the original *E. coli* strain used for infection. This identification was based on similarities with the original strain in O and K serotype, biochemical pattern, serum resistance, hemolysis, and attachment to urinary epithelial cells. Several bacterial isolates changed in O and K antigenicity, serum sensitivity, and virulence when remaining in the urinary tract during the long-term infection.

The fraction of weak O6, SA, and O6-negative but K13-positive strains among both urinary and renal isolates increased with time. The increased serum sensitivity of the bacterial isolates was accompanied by autoagglutinability of the bacteria.

These findings are in agreement with studies in humans in which urinary *E. coli* isolates from untreated individuals were able to persist in their O typability and became SA (15). An increased serum sensitivity of urinary bacterial isolates from untreated ABU patients was also reported (22), whereas strains isolated from patients with pyelonephritis were more resistant to the defense by depressing the virulence of the bacteria during their long-term presence in the urinary tract (15).

Serum antibodies to K13 were also produced, but only IgM, and no difference in occurrence was seen between healthy and pyelonephritic rats. The appearance of IgM anti-K antibodies alone agrees with earlier findings of Kaijser et al. (8), who recorded mainly IgM antibodies to *E. coli* K antigen in pyelonephritic patients.

Antibodies to lipid A were found in the group of rats with pyelonephritis or renal scars due to pyelonephritis or both. No relation was seen between high anti-O6 antibodies and occurrence of anti-lipid A antibodies. The results are in accordance with the findings in humans with UTI and progressive renal parenchymal reduction. Patients with radiologically confirmed renal scarring after acute pyelonephritis showed significantly higher IgG anti-lipid A antibody titers than did healthy individuals (5, 17). Increased anti-lipid A antibody titers have also been shown in patients with acute UTI and recurrences (25, 33). Lipid A, which can induce interstitial nephritis in dogs, has been suggested to be involved in the pathogenesis of renal damage due to UTI (25, 32).

### TABLE 4. Virulence of *E. coli* O6K13 isolated at various times postinfection from the urine of three rats as tested in the experimental ascending pyelonephritis model

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Time (mo) after onset of infection</th>
<th>Serotype*</th>
<th>No. of rats with pyelonephritis/total no. of rats infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>O6K13</td>
<td>6/20 (30)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>O6K13</td>
<td>8/19 (42)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>&quot;O6&quot;/K13</td>
<td>7/30 (23; <em>P &lt; 0.025)</em></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>&quot;O6&quot;/K13</td>
<td>14/28 (50)</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>&quot;O6&quot;/K13</td>
<td>10/29 (34)</td>
</tr>
<tr>
<td>Original strain</td>
<td></td>
<td>O6K13</td>
<td>35/58* (60)</td>
</tr>
</tbody>
</table>

* All five isolated strains had the same characteristics, biochemical pattern, serum bactericidal sensitivity, hemolysis, and attachment as the original *E. coli* O6K13H1 strain.

* Kolmogorov-Smirnov test.

* Concurrently tested controls (five experiments).
FIG. 3. Serum IgM (a), IgG (b), and IgA (c) antibody response to O6 LPS in E. coli O6K13H1-infected rats with no histological changes and no renal bacterial growth (○) and in rats with pyelonephritis and renal bacterial growth (○). The numbers above the diagrams represent the numbers of rats with antibody titers/total number of rats analyzed. Points represent median values.

TABLE 5. Anti-lipid A antibodies in serum from rats infected with E. coli O6K13

<table>
<thead>
<tr>
<th>Pyelonephritis</th>
<th>Time after infection</th>
<th>Total no. of positive rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-4 mo</td>
<td>8 mo</td>
</tr>
<tr>
<td>+</td>
<td>6/16*</td>
<td>4/8*</td>
</tr>
<tr>
<td>-</td>
<td>0/9</td>
<td>1/4*</td>
</tr>
</tbody>
</table>

* Two rats had IgG anti-lipid A antibodies and the other four had IgM antibodies.
+ Three rats had IgM antibodies and another had both IgM and IgG antibodies.
- Antibodies of both IgG and IgM classes.

ACKNOWLEDGMENTS

The skillful technical assistance of Helena Kahu, Helena Lomberg, Ingela Delgado, and Kerstin Larsson is very much appreciated.

This study was supported by grants from the Medical Faculty, University of Göteborg, the Swedish Medical Research Council (project 215), the Swedish Board for Technical Development (project EKB-U-614), the Ellen, Walter and Lennart Hesselman Foundation for Scientific Research, and Stiftung Volkswagenwerk (West Germany).

LITERATURE CITED


