Experimental Model of Corynebacterium renale Pyelonephritis Produced in Mice

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Corynebacterium renale type I (strain 115), $1.7 \times 10^7$ to $4.5 \times 10^7$ organisms, introduced intravenously into mice disappeared from the blood less than 24 h after inoculation and did not produce pyelonephritis. The same strain, $1 \times 10^7$ to $5 \times 10^7$ organisms, inoculated into the urinary bladder of mice was not recovered from the blood in any of the mice, but caused pyelonephritis accompanied by ureteritis and cystitis in 16 of 21 (76%) mice. Pyelonephritis and cystitis in mice were histopathologically similar to those found in cows. The antibody response was observed only in the mice with pyelonephritis or pyelitis, but not in those with only cystitis or in those without lesions, as found in cows. Similar diseases were produced in mice by C. renale types II and III but less frequently than by type I. It is suggested, therefore, that mice may be useful in the study of bovine C. renale infection.

Corynebacterium renale is the cause of pyelonephritis in cows. Some workers produced experimental pyelonephritis due to C. renale in mice by inoculating a large number of the organisms intravenously (6, 7), whereas others failed to produce pyelonephritis by inoculating the organisms intraperitoneally (4). Pyelonephritis due to C. renale in cows is known to be caused by penetration of the organisms from the lower urinary passages (1, 9). A model of the retrograde infection of C. renale has not been made in experimental animals.

In the present study, a retrograde infection was produced by inoculating C. renale into the urinary bladder of mice, and it was compared with the disease in cows.

MATERIALS AND METHODS

Mice. Female ddY-F mice, 6 to 7 weeks old, weighing 20 to 25 g, were used.

Bacterial strains. C. renale 115 (type I) was usually used. Strains of other serological types (11, 12), 92 RH (type II) and D-454 (type III), were also used.

Methods of inoculation. Organisms of each strain cultivated at 37°C for 1 day (2 days for strain D-454) on nutrient agar medium were suspended in saline and used for inoculation. Intravenous inoculation was done by injecting 0.02 ml of a suspension containing $1.7 \times 10^7$ to $4.5 \times 10^7$ organisms in a tail vein of the mice. Retrograde infection was done as follows. The mice were anesthetized with ether and forced to micturate, the abdominal wall was incised, and 0.02 ml of a suspension containing $10^7$ to $5 \times 10^7$ organisms was inoculated into the urinary bladder. No foreign body was placed in the bladder. The control mice were inoculated only with saline or a saline suspension of bacteria heated at 120°C for 15 min.

Bacterial examination. The mice were killed at various intervals between 30 min and 35 days after inoculation. The urinary bladder, ureters, kidneys, liver, spleen, ascites, and heart blood were examined for recovery of C. renale. Portions of these organs were inoculated on nutrient agar plates and incubated for 2 days at 37°C. Estimation of bacterial number in the blood was done as follows. A 0.2-ml volume of the blood was obtained from the femoral artery at 0.5, 2, 6, 12, 24, and 48 h after inoculation, and a serial 10-fold dilution of the blood was inoculated on nutrient agar plates. The urine for the bacterial culture was obtained at 2-day intervals and inoculated on nutrient agar plates. Quantitative bacterial cultures were done on the urine and the homogenates of the renal tissue. The homogenate was made with 20 volumes of saline solution, and then a serial 10-fold dilution of the homogenate was inoculated on nutrient agar plates. The colonies that developed were counted. Representative colonies of C. renale grown from the blood, urine, and tissue of each animal were identified as C. renale by immunodiffusion, as described previously (12). An attempt to isolate C. renale from normal mice was made from the urine, urinary bladder, ureters, kidneys, liver, spleen, lung, uterus, and heart blood.

Serological examination. A serum sample was obtained from each mouse after necropsy. The serum antibody titer was determined by an agglutination test, using heat-killed antigen prepared as described by Hiramune et al. (3). A serial twofold serum dilution (0.5 ml) was mixed with equal volumes of the antigen in test tubes. The tubes were kept at room temperature overnight, and the results were read. The titers were expressed as the reciprocal of the highest dilution showing definite aggluti-
nation. Antibody response was judged positive when the mice showed definite agglutination at a 1:20 or more serum dilution.

Pathological examination. The urinary bladder, kidneys, and other organs were examined for pathological changes. Tissues were collected, fixed in 10% Formalin, embedded in paraffin, sectioned, and stained by hematoxylin and eosin.

RESULTS

Detection of *C. renale* and antibodies against *C. renale* in normal mice. *C. renale* was not isolated from the urine, kidneys, and other organs of 25 normal mice that were selected randomly before use from a total of 250 mice. Antibodies against *C. renale* types I, II, and III were not detected in the sera of these mice.

Hematogenous infection. The mice intravenously inoculated with $1.7 \times 10^7$ to $4.5 \times 10^7$ organisms were killed from 2 h to 14 days after inoculation, and the distribution of the organisms in the mice was examined. None of the mice died or was moribund.

From the blood, from 5 to $1.3 \times 10^5$ organisms per ml were recovered in each two mice killed at 2 and 6 h after inoculation and one of the two mice killed at 12 h after inoculation. Thereafter, no organisms were recovered from the blood at 24 h (2 mice), 48 h (3 mice), and 3 to 14 days (total, 19 mice) after inoculation. From the liver and spleen, the organisms were recovered 24 and 48 h after inoculation, respectively, but not later. From the kidneys, the organisms were recovered from 4 of 6 mice from 2 to 12 h after inoculation and from 2 of 24 mice from 24 h to 14 days after inoculation. From the ascites and urinary bladder, no organisms were recovered throughout the period of the experiment.

The kidneys, ureters, and urinary bladder of the inoculated mice were examined histopathologically 2 to 14 days after inoculation. No remarkable changes were found in the cortex, medulla, papilla, pelvis, ureters, and urinary bladder in almost all of the intravenously inoculated mice. Mice killed 10 and 14 days after inoculation which showed a slight lymphocytic infiltration in the perivascular interstitial tissue in the cortex and medulla were the exceptions. In the control mice, no remarkable changes were found, and *C. renale* was not recovered.

Antibody response was positive without exception in intravenously inoculated mice after 3 days postinfection.

Retrograde infection. (i) No recovery of *C. renale* from the blood in the early stage of infection. A preliminary experiment was done to determine whether *C. renale* invaded the blood from the urinary bladder, particularly in the early stage of the retrograde infection. The $1.7 \times 10^7$ to $4.5 \times 10^7$ organisms inoculated into the urinary bladder of mice were not recovered from the blood; 0.2 ml of the blood obtained from the femoral artery of each two mice killed at 0.5, 2, 6, 12, and 24 h after inoculation, and the heart blood of a total of six mice killed on 48, 72, and 96 h after inoculation were negative in the recovery of *C. renale*. Recovery of *C. renale* in the early stage of the infection was negative in the liver and spleen but positive in the kidneys; the organisms were recovered from the kidneys in 40% of the mice killed within 24 h, and the rate increased to 100% at 48 and 96 h after inoculation.

(ii) Recovery of *C. renale* from the urinary organs. Recovery of *C. renale* from the urinary organs was examined in the following experiments, in which 67 mice were inoculated with $1.7 \times 10^7$ to $5 \times 10^7$, $1 \times 10^7$ to $5 \times 10^7$, and $1 \times 10^6$ to $5 \times 10^6$ organisms and were killed 4 to 35 days after inoculation. More than one-half of the mice affected with pyelonephritis died or were moribund 5 to 12 days after inoculation. The organisms were recovered only from the urinary organs. The recovery was positive until 21 days postinfection. The number of *C. renale* recovered from the kidneys varied from $10^{2.5}$ to $10^{7.7}$/g of tissue. The number of mice from which *C. renale* was recovered from the kidneys was 13 of 21 inoculated with $1 \times 10^7$ to $5 \times 10^7$ organisms, two of 26 inoculated with $1 \times 10^6$ to $5 \times 10^6$ organisms, and 3 of 20 inoculated with $1 \times 10^5$ to $5 \times 10^5$ organisms. Thus, *C. renale* appeared to reach the kidneys in most of the mice inoculated with $1 \times 10^7$ to $5 \times 10^7$ organisms, whereas the organisms disappeared from the urinary bladder without reaching these kidneys in many of the mice inoculated with less than $5 \times 10^6$ organisms.

Recovery of *C. renale* from the kidneys was accompanied by pyelonephritis or pyelitis. Recovery of the organisms from the urinary bladder was accompanied by cystitis. The mice without lesions were negative in the recovery of *C. renale*.

(iii) Excretion of *C. renale* into the urine. Eighteen of the 21 (86%) mice inoculated with $1 \times 10^7$ to $5 \times 10^7$ organisms excreted a large number of *C. renale* ($10^7$ to $10^8$/0.02 ml) into the urine 2 to 17 days after inoculation. Most of them shed the organisms continuously and showed hematuria. Less than one-half of the mice inoculated with less than $5 \times 10^6$ organisms excreted *C. renale* into the urine; continuous shedding was rarely seen, and hematuria
was seen in only some of them. The number of organisms in the urine of the mice inoculated with $10^6$ organisms was usually smaller than that of the mice inoculated with $10^7$ organisms. After 18 days postinfection, the number of C. renale excreted into the urine decreased remarkably.

Pyelonephritis was found almost exclusively in the mice that excreted a large number of C. renale, whereas it was scarcely found in the mice that excreted a small number of the organisms (Table 1).

### Table 1. Occurrence of pyelonephritis in relation to number of C. renale excreted into the urine

<table>
<thead>
<tr>
<th>Maximum no. of C. renale excreted in the urine (per 0.02 ml)</th>
<th>Pyelonephritis&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of mice&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>From $10^2$ to $10^6$</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>Less than $10^2$</td>
<td>1</td>
<td>42</td>
</tr>
</tbody>
</table>

<sup>a</sup> Histopathologically examined.

<sup>b</sup> Number of mice.

(iv) Pathological changes. The mice were divided into four groups depending on their lesions (Table 2). Acute pyelonephritis accompanied by ureteritis and cystitis was parti-

### Table 2. Lesions of urinary organs of mice in relation to inoculum size

<table>
<thead>
<tr>
<th>Lesions&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of mice&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute pyelonephritis, ureteritis, and cystitis</td>
<td>16 2 2</td>
</tr>
<tr>
<td>Chronic pyelitis, ureteritis, and cystitis</td>
<td>0 1 1</td>
</tr>
<tr>
<td>Cystitis alone</td>
<td>2 5 1</td>
</tr>
<tr>
<td>No lesions</td>
<td>3 18 16</td>
</tr>
</tbody>
</table>

<sup>a</sup> Histopathologically examined.

<sup>b</sup> Of the 16 mice that showed acute pyelonephritis, 2 showed only unilateral pyelitis accompanied by ureteritis and cystitis.

<sup>c</sup> Inoculum size.

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**Fig. 1.** Section of mouse kidney 5 days after inoculation of C. renale type I. Acute pyelonephritis with necrosis in the pelvis and the medulla adjacent to the pelvis (the lower part of the figure), polymorphonuclear infiltration, and congestion in the medulla is evident. The cortical tissue is normal. Hematoxylin and eosin; ×120.

**Fig. 2.** Section of the urinary bladder 14 days after inoculation of C. renale type I. Infiltration of polymorphonuclear cells and lymphocytes and necrosis are found in the mucosa. Hematoxylin and eosin; ×300.
larly frequent (16 of 21 mice (76%)) in the mice inoculated with $1 \times 10^7$ to $5 \times 10^7$ organisms, but was infrequent (4 of 46 mice (9%)) in the mice inoculated with less than $5 \times 10^6$ organisms. Chronic pyelitis accompanied by ureteritis and cystitis was found in only two mice that were inoculated with less than $5 \times 10^6$ organisms. Cystitis alone was seen in eight mice. No lesions were found in many of the mice inoculated with less than $5 \times 10^6$ organisms, but lesions were found in a few of the mice inoculated with $10^7$ organisms.

The kidneys from which C. renale was recovered were swollen and congested. The renal pelvis was dilated with a slimy exudate. Pyelonephritis with polymorphonuclear infiltration was seen in these kidneys (Fig. 1). Necrosis was found in the papilla and in the pelvis. The cortical tissue was usually normal.

The bladders from which C. renale was recovered were thickened and covered with a slimy secretion mixed with cell debris and fibrin, and the mucosa was ulcerated superficially (Fig. 2). Hemorrhaging sometimes occurred in the bladder wall. Epithelial degeneration and ulceration of the mucous membrane of the bladder were conspicuous.

In the control mice, no remarkable changes were found, and C. renale was not recovered.

(v) **Antibody response in mice.** The antibody response against C. renale was positive in all of the mice affected with pyelonephritis or pyelitis (Table 3). No antibody response was seen in the mice affected with cystitis alone, in those without lesions, or in the control mice.

(vi) **Renal infection due to C. renale type II and III in mice.** Pyelonephritis and cystitis were produced by C. renale types II and III but only at a low rate. The lesions produced by type II were very slight, whereas those produced by type III were as severe as those produced by type I.

**DISCUSSION**

In the present study, pyelonephritis in mice was produced by retrograde infection with C. renale. It is suggested from histopathological findings that the organisms inoculated into the urinary bladder reached and grew in the pelvis first and then invaded the medulla. The retrograde infection of C. renale in the mice was similar in many respects to that in cows. Hematuria was observed in both cows and mice. C. renale was recovered only from the urinary organs in both animal species. The recovery rate of the organisms from the urine and kidneys was as high in mice as in cows (2). Renal lesions of the mice were similar to those of the cows experimentally (2) and naturally (4) infected with C. renale. The antibody response was always accompanied by pyelonephritis but was not accompanied by cystitis alone or by any lesions in both the cows (2, 3) and the mice. The main difference was that the infection was essentially chronic in cows but acute in mice. It is interesting to note that the virulence of C. renale types I, II, and III was different in mice compared with that in cows (2). The experimental model of C. renale pyelonephritis in mice is helpful, therefore, in studies of the infection in cows.

Jones and Little (4) produced pyelonephritis in cows by inoculating C. renale into the urinary bladder but not by inoculating the same organisms intravenously. We obtained the same results in the experiment with mice. By intravenous inoculation of C. renale into mice, pyelonephritis was produced by Lovell and Cotchin (7) but not by us. The difference may be due to virulence of the bacterial strains and the strains of mice.

The pyelonephritis found in mice was generally acute, whereas in cows was chronic (2). The difference may be due partly to the smaller-sized urinary bladder of the mice. In mice, the organisms may be immediately forced up the ureter into the kidney by pressure of the inoculated bacterial suspension on the bladder. In contrast, in cows the bacterial suspension may diffuse into the bladder, and the bacteria may not be forced up into the kidney. The number of inoculated organisms per unit volume of urine in the urinary bladder was larger in mice than in cows. The occurrence of pyelonephritis in mice was ultimately associated with the number of C. renale in the urine. A similar

| Table 3. Antibody response and its relation to pyelonephritis or pyelitis |
|---------------------------------|-----------------|-----------------|-----------------|
| Lesions*                        | Antibody response$^a$ in mice inoculated with organisms: |
|                                 | $1 \times 10^7$ to $5 \times 10^7$ | $1 \times 10^8$ to $5 \times 10^8$ | $5 \times 10^8$ |
| Pyelonephritis or pyelitis      | $13/13^c$       | $1/1$            | $2/2$           |
| Cystitis alone                  | $0/1$           | $0/5$            | $0/1$           |
| No lesions                      | $0/3$           | $0/10$           | $0/11$          |

$^a$ Histopathologically examined.
$^b$ The antibody response in mice was examined on and after the day 5 postinfection. The antibody response was judged positive when the mice showed definite agglutination at 1:20 or more serum dilution.
$^c$ The numerator indicates number of mice whose antibody response was positive; the denominator denotes number of mice examined.
finding was reported for human pyelonephritis due to *Escherichia coli* (5, 8, 10).

Only a few investigations were done on the host-parasite relationship in *C. renale* infection. Further studies on the infection of *C. renale*, especially on the lack of antibody response in the stage of cystitis, and on the mechanisms of the ascending of *C. renale* from the bladder to the kidney, can be done by using the experimental model in mice given in the present study.

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**LITERATURE CITED**