Bactericidal Antibody Response to *Pseudomonas aeruginosa* by Adults with Urinary Tract Infections

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In this investigation we found that adults with upper urinary tract infections caused by *Pseudomonas aeruginosa* produced serum antibodies with bactericidal activity against the bacterium. Seventeen of 20 infected adults showed bactericidal activity with a titer range of 1:10 to 1:10,000.

Urinary tract infections (UTI) are most often found in females and children. Lower UTI are much more common than upper UTI (2), and most UTI are caused by *Escherichia coli*. *Pseudomonas aeruginosa* is increasingly being found to cause UTI (5) and accounts for 9% of UTI (1). Since UTI may occur even during antibiotic therapy, the host's protective immune response would be important not only during an active infection but in any reinfection. Pazin and Braude (7) demonstrated that immobilizing antibodies found in human serum represent a protective immune response which may limit bacterial invasion of the urinary tract by the antibodies acting as opsonins to increase phagocytosis. The study reported here has found a bactericidal antibody response to *P. aeruginosa* in 17 sera from 20 adult patients with active upper UTI.

Fresh sera were obtained for bactericidal testing prior to antibiotic therapy from 12 male and 8 female adults (aged 24 to 83) having active lower and upper UTI caused by *P. aeruginosa*. A subculture in tryptic soy broth (Difco, Detroit, Mich.) of the *P. aeruginosa* strain isolated from each patient's urine was used in the bactericidal assay. Control sera for possible bactericidal activity against *P. aeruginosa* were obtained from five normal adult males (aged 25 to 48) who did not have UTI.

Bactericidal titers of the human sera were determined by using a modification of the technique described by Kwapiszni (3). Ten-fold serial dilutions of *P. aeruginosa* cultures were made in sterile 0.85% NaCl. Two-tenths milliliter of each bacterial dilution and 0.2 ml of each patient's serum were pipetted separately into sterile tubes (15 by 100 mm) and done in triplicate. Control tubes received 0.2 ml of each dilution of bacteria and also 0.2 ml of sterile 0.85% NaCl. Both test and control tubes were incubated in a 37°C water bath for 1 h, and the dilutions were then plated by mixing with molten Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.). The agar was allowed to solidify, and the plates were then incubated at 37°C for 24 h before observation.

Colonies of the test and control plates were separately counted to obtain the total plate count. The number of colonies from the control plates were compared with number of colonies of the test plates at each culture dilution to obtain the percentage of bacteriolysis. Test plate counts were considered to be positive for serum bactericidal activity at the dilution in which 50% or more of the bacterial colony growth was inhibited when compared with control plates of the same dilution (3).

One-half milliliter of one male adult serum, which had been stored at -22°C and had a 1:10,000 bactericidal titer, was applied to a Sephadex G-200 molecular exclusion gel column having a 50-ml bed volume. The eluate was collected in 2-ml samples, and the optical density of each tube was determined at 280 nm with a spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). Tubes of the ascending portion of the 19S globulin peak and tubes of the descending portion of the 7S globulin peak were separately pooled. The protein concentrations of the 19S and 7S globulin fractions were determined at $E_{280}^{1\%} = 15$ (4). For bactericidal testing, the 19S and 7S globulin fractions both had protein concentrations of 1 mg/ml.

The 19S and 7S globulin fractions were both tested for bactericidal activity, but none was found. Because complement plays an important role in the bactericidal response and certain components were probably lost either during storage or by gel filtration, 0.2 ml of human complement (provided by Stratton Blood Bank, Methodist Hospital, Memphis, Tenn.) was added to each milliliter of 19S and 7S globulin fraction. The globulin fractions were then re-
tested for bactericidal activity.

The five uninfected adult males used as controls showed no bactericidal activity against *P. aeruginosa* (Table 1). Ten males and 7 females or 17 of the 20 actively infected adults with positive bactericidal titers were diagnosed by their attending physicians as having pyelonephritis or another upper UTI. The three infected adults (two males and one female) with negative bactericidal titers were diagnosed by their attending physicians as having lower UTI.

The 19S and 7S globulin fractions initially showed no bactericidal activity. After the addition of human complement, the 7S fraction showed positive bactericidal activity, with a 1:10 bactericidal titer, but the 19S fraction remained negative.

In this study of UTI by *P. aeruginosa*, a bactericidal response was demonstrated in 85% or 17 of the 20 infected adults tested (Table 1). A bactericidal titer range of 1:10 to 1:10,000 was found with these patients (Table 1). By gel filtration chromatography, the 7S immunoglobulin G (IgG) fraction showed bactericidal activity at a titer of 1:10 after addition of complement. The 19S IgM fraction remained negative after addition of complement. Young (8) stated that both IgG and IgM antibodies can function as opsonins, but it was not clear which one was more closely associated with protection. The results of the present study showed a protective bactericidal response by 7S IgG. However, it is possible that the 19S IgM may have been too greatly diluted during the Sephadex separation and thus the 19S bactericidal response was not detectable.

The demonstration in this investigation of positive bactericidal antibody activity by human sera appears to correlate well with the presence of upper UTI and pyelonephritis (Table 1). It has been postulated that prolonged antigen stimulation of the renal parenchyma is responsible for serum antibody production (6). Thus, the presence of a bactericidal immune response in patients with upper UTI may by due to the prolonged antigenic stimulation of the renal parenchyma (6) and may provide protection from a reinfection of *P. aeruginosa*.

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**LITERATURE CITED**


**Table 1. Bactericidal serum antibody response exhibited against *P. aeruginosa* by 17 patients with upper UTI**

<table>
<thead>
<tr>
<th>Test group</th>
<th>No. of sera</th>
<th>Bactericidal titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Lower UTI</td>
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