Immune Response in Urinary Tract Infection Determined by Radioimmunoassay and Immunofluorescence: Serum Antibody Levels Against Infecting Bacterium and Enterobacteriaceae Common Antigen

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A solid-phase radioimmunoassay (RIA) procedure was compared with the indirect fluorescent antibody (IFA) test in a serological study of 76 female adults with urinary tract infections. Relative serum antibody activity was determined against patients' homologous infecting enterobacteria by RIA and IFA and against heterologous enterobacterial common antigen (Escherichia coli O14) by RIA. There was marked correlation between results of the IFA and RIA methods using the homologous system; 22 of 51 patients (43%) with pyelonephritis had significantly elevated serum antibody activity by both IFA (titers ≥512) and RIA (binding ratio ≥2.0) when compared with normal serum controls; three had significant antibody activity detectable by RIA only. Eighteen (72%) of 25 patients with pyelonephritis had RIA binding ratios of ≥2.0 against their homologous bacterial isolates and the enterobacterial common antigen; an additional 6 patients had binding ratios of ≥2.0 against the antigen only. All 25 patients with cystitis had low serum antibody levels by IFA and RIA when tested against their own isolate as well as enterobacterial common antigen. The RIA procedure was objective, quantitative, and less tedious to perform than IFA.

We have used indirect immunofluorescence to study the immune response in patients with urinary infections (9). The serum antibody titers were determined against the patients' own infecting organism. We have also used direct immunofluorescence to detect the presence of antibody-coated bacteria in urine sediment, a finding associated with kidney infection rather than bladder infection (9–11). Based on the results of these tests, patients could be divided into three groups: (i) those with pyelonephritis, positive urine sediment fluorescent antibody (FA) tests, and elevated serum antibody; (ii) those with pyelonephritis, positive urine sediment FA tests, and normal titers of serum antibody; and (iii) those with cystitis, negative urine sediment FA tests, and normal titers of serum antibody. The finding of an elevated serum antibody titer is diagnostically significant because it indicates kidney infection (9). The indirect fluorescent antibody (IFA) procedure we routinely use to measure serum antibody against antigens on the surface of the bacterial cell is a sensitive method of detecting primary antigen-antibody reactions; however, the procedure is tedious and time-consuming because it requires testing serial dilutions of each serum. Recently Sanford and Smith (7) used a solid-phase radioimmunoassay (RIA) procedure for measuring antibacterial antibody in human sera; they found that antimicrobial antibody could be measured by using a single dilution of serum rather than serial dilutions.

The object of our present study was to determine whether the rapid RIA procedure could be used in place of the indirect immunofluorescence method for measuring serum antibody levels in clinically well-defined groups of patients known to have urinary tract infections. In addition, we wanted to compare RIA results of testing patients' serum antibody activity against their own infecting organisms with the results of tests against enterobacterial common antigen (ECA).

MATERIALS AND METHODS

Clinical specimens. Blood specimens for serum antibody studies were collected from 76 female patients within a 2-week period during an episode of urinary tract infection and from 14 women without past history of urinary tract infection for controls.
Urine specimens were collected from the 76 patients during the episode of active infection and before antibiotic therapy. The urines were either catheter or midstream specimens taken after thorough cleansing. The specimens were cultured quantitatively by the calibrated-loop technique, and the bacterial isolates were identified by standard bacteriological and biochemical procedures.

**Diagnosis.** The diagnosis of chronic pyelonephritis was based on results of urine cultures, the patient’s clinical course, characteristic changes on intravenous pyelography, impairment in renal function, and direct localization by ureteral catheterization or the bladder washout method when possible. The diagnosis of acute pyelonephritis was based on the history of fever, chills, and flank pain and the finding of positive urine cultures. Nausea and vomiting, as well as frequency, urgency, and dysuria, were frequent symptoms; costovertebral angle tenderness was always found. The clinical diagnosis of cystitis was based on the history of frequency, urgency, dysuria, suprapubic pain, and significant bacteriuria (≥100,000/ml); fever, chills, and flank pain were absent. Renal function and radiological studies were normal.

**Localization of the anatomic site of infection.** Urine specimens were tested for the presence of antibody-coated bacteria by direct immunofluorescence using fluorescein-conjugated horse antiserum to human globulin (Roboz Surgical Instrument Co., Washington, D.C.). Antibody-coated bacteria are associated with kidney infection rather than bladder infection (9).

**IFA tests.** Titers of serum antibody to the patient’s own infecting bacteria were determined by indirect immunofluorescence; both heated and unheated whole-cell preparations were used. Saline controls and normal human serum controls were included in each test (9). RIA. Relative antibody activity was determined for each serum against the patient’s own infecting organism and ECA using a solid-phase bead RIA system (7, 8). Antigens used in the RIA tests were sonic extracts prepared from fresh 18 h subcultures of bacteria as previously described (7). ECA was prepared from a stock culture of Escherichia coli 014:K7:NM (Center for Disease Control no. SV441l-41; Atlanta, Ga.). Sonic extracts were aliquoted and stored at −70°C; before use, each antigen extract was adjusted to a concentration of 0.1 mg of protein per ml with phosphate-buffered saline (0.01 M phosphate in 0.15 M saline) (pH 7.2). Details of the RIA procedure have been reported (7, 8). Briefly, duplicate ferromagnetic beads were coated with bacterial antigen; beads were rinsed with 1% bovine serum albumin, then placed in individual wells, each containing 0.2 ml of the patient’s serum diluted 1:1,000 in 1% bovine serum albumin. After incubating for 18 h at 4°C, beads were rinsed in deionized water and placed in wells containing 0.2 ml of 125I-tagged anti-human immunoglobulin for 4 h at 22 to 25°C. Beads were washed, placed in tubes, and measured for activity in a Beckman gamma counter. Diluent and normal serum controls were included in each test. The goat antiserum used in the RIA procedure was purified in our laboratory by affinity chromatography and labeled with 125I by the chloramine T method of Daugherty et al. (3) using 1- to 5-mg protein concentrations of antibody and 2 mCi of Na125I (Amer sham, Arlington Heights, Ill.). Results of RIA tests were calculated as follows: corrected counts per minute (CPM) were obtained by subtracting the background CPM, obtained in the diluent control, from the mean CPM, obtained from duplicate tests of each patient and control serum versus each bacterial antigen. The relative antibody activity of each test serum against a particular antigen is expressed as the binding ratio, which was determined by the following formulas: (i) binding activity = corrected CPM of test or control serum/corrected CPM of a normal serum control with the lowest activity in CPM; (ii) binding ratio = binding activity of test serum/mean binding activity of the normal serum controls.

**RESULTS**

Urine specimens from 76 patients with urinary infections were tested by FA for the presence of antibody-coated bacteria. The urine FA test was positive in 51 of the patients (pyelonephritis) and negative in 25 (cystitis). Serum specimens from the 76 patients were tested by IFA to determine antibody titers against the patient’s own infecting organism. The 51 patients who had antibody-coated bacteria in their urines were divided into two groups based on their serum antibody titers; 22 had elevated titers of ≥512, and 29 had titers of <512. Antibody titers in this latter group were comparable to those of the 25 patients with cystitis, who had negative FA tests for the presence of antibody-coated bacteria in the urine. A serum titer of <512 is considered to be within the normal range (9).

Results of RIA studies are shown in Fig. 1. There was a marked correlation between the results of RIA and IFA tests (coefficient of correlation = 0.69). RIA binding ratios ranged from 2.3 to 6.6 (mean 3.5) in sera from 22 patients who had IFA titers of ≥512 against their own infecting organism. Of the 54 patients with IFA titers of <512, 51 (94%) had RIA binding ratios of <2.0. Results of the two methods did not agree for three patients with pyelonephritis whose serum titers were considered low by IFA (<512) but whose serum binding ratios were elevated by RIA (2.3, 2.4, and 2.6). Each of the 25 patients with cystitis who had negative urine sediment FA tests and serum antibody titers of <512 had RIA serum binding ratios of >2.0 (mean 1.0, range 0.4 to 1.9).

Table 1 compares the bacterial isolates from the three groups of patients and shows that elevated RIA binding ratios were associated with a variety of gram-negative rods. E. coli was isolated from each of the three patients with pyelonephritis whose serum titers were within the normal range by IFA but whose binding ratios were elevated by RIA.
patients by RIA for antibody against their own infection, pyelonephritis, positive urine sediment FA tests, and ranged from patients with infections.

Serum samples from the 76 patients and 14 controls were tested for antibody against ECA using RIA. Results for the 51 patients with pyelonephritis are summarized in Table 2; the total percentage of patients with elevated antibody levels against their own infecting organism (49%) was close to the percentage of patients with elevated antibody levels against ECA (47%). Even though the percentages of the two groups were equivalent, the patients in each group were not necessarily the same. Individual test results were in agreement for 38 (75%) of the 51 parallel RIA tests comparing homologous antigen with ECA. RIA binding ratios for the 25 patients with cystitis were ≤1.1 in tests with ECA (mean 0.6, range 0.2 to 1.1). Binding ratios for patients with cystitis were significantly lower (P = 0.01) than those for 14 control individuals without history of urinary infection (mean 1.0, range 0.5 to 1.4).

DISCUSSION

To determine the role of serum antibodies in patients with kidney and bladder infections, a technique is needed which is sensitive and specific, allows objective interpretation, can be used to measure antibody activity of numerous sera against numerous antigens, and can be used to detect antibodies of all classes. The present study established the usefulness of a solid-phase RIA procedure which seems to meet these criteria. Sera from 76 patients with urinary tract infections were tested for relative antibody activity against the patient's own infecting organism using both RIA and IFA. There was a marked correlation between results obtained by the two methods (Fig. 1). In fact, disagreement occurred in only three cases, in which antibody levels were elevated by RIA but not by IFA. These discrepancies may be explained by inherent differences between the two test systems due to the state of antigen. The IFA test detects antibody to surface antigens of whole bacteria. However, crude bacterial sonic extracts rather than whole cells are used in the RIA test, and antigens in the extract nonspecifically adsorb to the solid-phase ferromagnetic beads, presumably the extracts contain a mixture of both ex-

![Figure 1](http://jcm.asm.org/)  
**FIG. 1.** Results of testing serum samples from 76 patients by RIA for antibody against their own infecting bacterial isolates. □, 22 patients with pyelonephritis, positive urine sediment FA tests, and serum antibody titers of ≥512 by IFA; ■, 29 patients with pyelonephritis, positive urine sediment FA tests, and serum antibody titers of <512 by IFA; ◇, 25 patients with cystitis, negative urine sediment FA tests, and serum antibody titers of <512 by IFA.

![Figure 2](http://jcm.asm.org/)  
**FIG. 2.** Results of RIA testing of eight control serum samples for antibody activity against each of the 76 bacterial strains isolated from patients with urinary infections (608 tests).

**Table 1.** Patients with elevated RIA binding ratios grouped according to the kind of bacteria causing their urinary infection

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Urine sediment FA test</th>
<th>Serum antibody titer by IFA</th>
<th>Patient's isolates*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
<td>Klebsiella pneumoniae</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>Positive</td>
<td>≥512</td>
<td>9/9</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>Positive</td>
<td>&lt;512</td>
<td>3/24</td>
</tr>
<tr>
<td>Cystitis</td>
<td>Negative</td>
<td>&lt;512</td>
<td>0/18</td>
</tr>
</tbody>
</table>

* Number of patients with a serum RIA binding ratio of ≥2.0 against their own infecting organism/number of patients tested.
TABLE 2. Results of testing serum samples from 51 patients with pyelonephritis by RIA for antibody levels against their own infecting organisms and ECA

<table>
<thead>
<tr>
<th>IFA titer</th>
<th>Own infecting organism</th>
<th>ECA</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥512</td>
<td>22/22 (100)</td>
<td>15'/22 (68)</td>
</tr>
<tr>
<td>&lt;512</td>
<td>3/29 (10)</td>
<td>9'/29 (31)</td>
</tr>
</tbody>
</table>

Total 25/51 (49) 24/51 (47)

*Results expressed as number of patients with RIA binding ratio ≥2.0/number tested (percentage).

Patients’ isolates included: E. coli (9), K. pneumoniae (3), P. mirabilis (1), Enterobacter agglomerans (1), and Citrobacter diversus (1).

Patients’ isolates included: E. coli (7), K. pneumoniae (1), and P. mirabilis (1).

ternal and internal bacterial cell components. Possibly, antibody detected by RIA in the sera of these three patients reacted with antigens not available for reaction in the IFA test. In addition, discrepancies between IFA and RIA test results may be explained by differences in the sensitivity of the two test systems. The solid-phase RIA system has been shown to detect quantitative differences between sera that were not detected by IFA; the system is said to be at least 30-fold more sensitive than IFA (7). Thus, it is possible that the serum antibody titers in these three patients were sufficiently high to be detected by RIA but not by IFA.

In any serological study of patients with urinary tract infections, each patient’s serum must be tested against the patient’s own infecting organism(s); because this requires numerous antigen preparations, the procedure is time consuming. Ideally one should replace the numerous homologous antigens with a single standard antigen to be used in studying the serological responses of patients to gram-negative urinary pathogens. One possible standard antigen, the ECA, was described by Kunin et al. (5) and recently reviewed by Makela and Mayer (6). The ECA is shared among bacteria belonging to the family Enterobacteriaceae. A limited number of studies, especially pediatric, have been reported on the humoral response to ECA in patients with urinary tract infections. Whang and Neter (12) found persistently elevated levels of antibody against ECA in serum samples from five of six patients followed over a period of 1 year. Although their report seemed promising, subsequent studies did not seem to substantiate the association between infection and elevated levels of ECA antibodies. Sera from 22 children with acute pyelonephritis were tested by Andersen (2) for antibodies against their homologous infecting organisms and ECA. He found relatively high titers against ECA in two patients who frequently suffered from relapsing pyelonephritis; in the remaining group, antibody titers were either slightly elevated (two patients) or not elevated at all. Diaz and Neter (4) reported that of 50 children with acute or chronic urinary tract infections, 92% had elevated titers against their homologous organisms, while only 18% had elevated titers against ECA. Thus, the diagnostic importance of the antibody response against ECA in patients with urinary tract infection is still unknown. It appears that the primary limitation lies in the serological methods commonly used to determine antibody activity. Hemagglutination and hemagglutination-inhibition, which have been used most frequently, are known to favor the detection of immunoglobulin M antibodies and to underestimate the amount of immunoglobulin G antibodies (1).

To overcome this methodological problem, we used the solid-phase RIA procedure to determine anti-ECA activity in the sera from 76 patients and 14 controls; a sonic extract of E. coli O14 was used as the source of ECA. The test was successful in detecting elevated levels of antibody to ECA in the sera from 18 to 25 patients (72%) who had elevated levels of antibody against their own infecting organisms as determined by RIA (Table 2). In addition, the test also identified six patients who had elevated levels of antibody against ECA but not against their own infecting organisms. It is possible that the anti-ECA activity in these six patients reflects a previous episode of pyelonephritis with a different organism. These antibodies may persist at significant levels between episodes of infection, or they may be preferentially stimulated during an active infection. One intriguing observation was made in the group of 25 patients with cystitis. The level of their antibody activity against ECA was significantly lower than the normal levels found in sera from the control group. Whereas these results may reflect the state of immunocompetence of cystitis patients, it would be premature to speculate on the significance, if any, of this observation.

In conclusion, the RIA procedure is a rapid and simple system which is easily adapted for serological study of patients with urinary tract infections. In addition, the system seems potentially useful in exploring the diagnostic significance of anti-ECA antibodies, which are commonly found in the sera of patients with pyelonephritis.

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