ANTIBODIES IN ESCHERICHIA COLI URINARY TRACT INFECTION

D Fries, F Delavelle, D Mathieu, L Jacques, L Renault

Hôpital Paul Brousse, Villejuif, France

Summary

In 105 adults with E.Coli urinary tract infections, IgG coated bacteriuria was found in 8/9 with acute pyelonephritis (PN), 17/20 with chronic PN, and in only 2/76 with lower UTI. IgA was present in 66% of PN, but IgA secretory piece in less than 10%. These urinary IgG antibodies were, at least in part, synthesised in the kidney because serum IgG antibodies were detected by indirect immunofluorescence in only half the patients.

O6, O18, O22 E.Coli serotypes were the three most frequently found 0 groups, but their prevalence in PN is not significant.

The immunology of urinary tract infection (UTI) is still a subject of little interest in adult nephrology; but antibody production is a well-characterised event in pyelonephritis (PN), the study of which seems to be the best indirect procedure for localising the site of UTI. We have analysed the production of urinary and humoral antibodies, and their correlation with E.Coli serotypes in patients with E.Coli UTI.

Patients and Methods

Patients

This study concerns 105 adults (mean age 46 years) with a significant E.Coli bacteriuria (bacterial count $\geq 10^5$ ml). Ninety-one of these patients were women, urine specimens being collected by urethral catheterisation. Patients with surgical ureteric drainage, or men with prostatitis were excluded.

The diagnosis of upper UTI, i.e. PN, was made in 29 patients (9 acute PN, 20 chronic PN), according to clinical, biological and radiological criteria. The diagnosis of lower UTI was made in 76 patients (43 cystitis, 33 asymptomatic bacteriuria) of whom 66 were women.
Urinary antibodies

The technique for detecting antibody-coated bacteriuria (ACB) was that described by Thomas\(^3\), using fluorescein-conjugated rabbit antihuman IgG, IgA, IgM and IgD, C\(^3\), albumin and fibrinogen sera (Behring); in 11 patients with PN, an anti IgA - secretory piece serum was employed. The controls consisted of (i) washed sub-cultured bacteria on which antisera will not fix and (ii) the absence of fluorescence when a non-conjugated antiserum has been previously introduced. Tests were only considered as positive when a) at least 25\% of the bacteria were fluorescent, without taking into account the strength of the fluorescence; b) There is fluorescence with anti-IgG. The cases with IgA coating alone were excluded\(^4\).

Humoral antibodies

Two methods were employed: a) the passive haemagglutination (PH) technique, described by Neter\(^5\), in all 105 patients, a titre \(\geq 1/160\) being accepted as positive; b) the indirect immunofluorescence technique, described by Schmidt\(^6\), in 51 patients.

E. Coli Serotypes

E.Coli were grouped by direct bacterial agglutination: antisera were prepared in rabbits with O\(_1\) to O\(_{153}\) somatic antigen (From Dr Orskov, collaborative centre for reference and research on Escherichia, WHO, Copenhagen).

Results

Urinary antibodies (Table I)

a) Upper UTI: IgG-coated bacteria were found in the urine in 8 out of 9 cases of acute PN and 17 out of 20 with chronic PN. IgA was detected on the bacterial walls in 66\% of urines from PN but IgM in only 3 cases. IgA secretory piece was sought in 11 patients with PN, but a positive result was found in only one.

b) Lower UTI: only two out of 76 cases were positive for IgG: both had asymptomatic bacteriuria (1 diabetic, 1 focal glomerulonephritis).

c) Albumin, fibrinogen, IgD and C\(^3\) were never detected on the bacterial wall.

Humoral antibodies

a) Passive haemagglutination: Table I shows the poor correlation between humoral antibodies and the renal site of infection. It is interesting to note that the only positive case in lower UTI was the patient who was ACB positive with
a focal glomerulonephritis.

b) Immunofluorescence: Serum antibodies were detected in 3 out of 4 cases of acute PN: IgG and IgA in 2, IgG alone in one. ACB were present in the 3 cases, serum PH in 2. The negative case was passive haemagglutination negative, but ACB positive.

In chronic PN, half of the patients had no detectable antibodies either by IF or by PH. The six other cases with serum antibodies detected by IF also had an IgG positive bacteriuria: in 2, both IgG and IgA antibodies were detected and were also detected by PH; the 4 other cases had only IgG antibodies and all of them were negative by PH. In our experience, serum antibodies are detected by PH only when IgA antibodies are present.

In lower UTI, serum IgG antibodies were detected in only 3 out of 35 cases: these 3 cases were negative by PH and ACB.

**TABLE I. Urinary and Serum Antibodies (Passive Haemagglutination) in Upper and Lower UTI**

<table>
<thead>
<tr>
<th></th>
<th>Urinary IgG Antibody Positive</th>
<th>Serum Antibodies Passive Haemagglutination Positive</th>
</tr>
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<tbody>
<tr>
<td>Acute PN 9</td>
<td>8/9</td>
<td>4/9</td>
</tr>
<tr>
<td>Chronic PN 20</td>
<td>17/20</td>
<td>3/20</td>
</tr>
<tr>
<td>Lower UTI 76</td>
<td>2/76</td>
<td>1/76</td>
</tr>
</tbody>
</table>

**E. Coli Serotyping**

Serotyping performed in 72 patients was repeated in cases with persistent bacteriuria. A total of 81 strains were studied. Twenty-eight of the eighty-one were unidentified E. Coli strains (34.5%). Rough strains were present in six (7.5%). With the 163 specific antisera systematically employed for typing, 20 strains were identified (47/81; 58%). Three groups were most commonly (24/81) encountered: O6 (9 strains), O18 (9 strains), O22 (6 strains).

Table II shows the correlation between E. Coli groups and the site of UTI. In 28% of the 81 cases studied, the infection was localised to the kidney; the percentage with PN is approximately the same for the 3 most common E. Coli groups (29%), and for the non-typable strains (32%); it differs from the rough strains (66%) and from the 17 other serotypes identified (14%).
Discussion

Thomas\textsuperscript{3} has shown that in renal infection, bacterial walls are coated by antibodies detected by means of fluorescein-conjugated anti-immunoglobulin antisera, and that in lower UTI this fluorescence test is negative. Several reports\textsuperscript{3,7,8,9} have confirmed that this new method is a sensitive, reliable and non-invasive technique for localising the site of infection. In this series of E. Coli UTI, we have observed 4 negative cases in 29 with pyelonephritis. As reported previously\textsuperscript{2}, two of them are doubtful, and the percentage of 'false negatives' may be estimated to be below 10\% (2/27). The bladder wash-out technique, that is held by many to be the best criterion for localising the site of UTI, gives a higher proportion of inaccuracies — above 10\% for Fairley himself\textsuperscript{10} and 43\% in a recent well-documented series of 14 acute PN in childhood\textsuperscript{11}.

Serum antibodies detected by passive haemagglutination were found in 24\% of PN (4/9 acute PN, 3/20 chronic PN). In our hands agglutination techniques gave even poorer results (unpublished data). In the literature, results differ from one series to another, the best correlation being obtained in acute PN in children. In a series of 111 adults with chronic PN, serum antibodies were detected by PH in 12.6\% and by agglutination in 16.5\% \textsuperscript{6}.

Indirect IF has rarely been employed in the study of the serum antibody response to UTI. In 3 patients with chronic PN (2 E. Coli, 1 Klebsiella) Vosti\textsuperscript{12} has found IgG antibody in each patient and IgM and IgA in 2 of them. Thomas\textsuperscript{3} detected IgG in 70\% of acute and chronic PN, Schmidt in 63\% of chronic PN. In our series, IgG antibodies were detected in 56\% of PN, and 8.5\% of lower UTI.

Urinary antibodies against E. Coli 0-antigen belong essentially to the IgG class. This is in accordance with the well-established fact that in PN, serum antibodies are IgG\textsuperscript{1}. In experimental E. Coli PN, IgG is also the predominant immunoglobulin synthesised in the kidney\textsuperscript{13}. The production of IgG, not IgM, is in favour of the persistence of antigen in the renal tissue, a recurrent infection due to a new strain, or to another E. Coli serotype enhancing the antibody response.

In patients with UTI, IgA increases in the urine, and has been shown to have antibody activity against the somatic antigen\textsuperscript{14}. But the production of IgA is much weaker than IgG, either in experimental or human PN\textsuperscript{13}. Our results fit quite well with these data. Among 9 patients with PN with serum IgG antibodies, IgA serum antibodies were found in 4, and urinary IgA antibodies in two-thirds. With the enzyme-linked immunosorbent assay, secretory IgA has been found in experimental pyelonephritic kidneys\textsuperscript{13} and in the urine of human UTI\textsuperscript{14}. In our series, IgA secretory piece was found in only one of 11 with PN, but larger series need to be studied.

Our results are in accordance with the observations that IgM antibodies seem to play a modest role in the immunological response to UTI\textsuperscript{1}: there were no IgM antibodies in the serum of our patients, and urinary IgM antibodies in only three of 29 with PN.

In about half of the patients, serum antibodies, synthesised in spleen and
lymph nodes, correlate well with urinary antibodies. It is conjecture that urinary antibodies come from serum; glomerular lesions or impaired tubular reabsorption could explain the passage of IgG in urine. But in half of the patients, these urinary antibodies, not detected in the serum, can be explained only by increased local synthesis in the kidney itself, assuming the validity of the ACB test in localising the site of UTI.

In this series, E. Coli typing showed an unusual frequency of O\textsubscript{22} serotype, that is not listed among the '8 most frequent 0-groups' reported by others\textsuperscript{14}. Groups $0_6$, $0_{18}$, $0_{22}$ represent 51% of the typed E. Coli strains occurring in our series, whereas the '8 most common 0-groups' would represent no more than 57.5%. The use of a pool of these 3 antigens would render E. Coli typing easier in our area.

A predilection for some E. Coli types to induce pyelonephritis is not clearly shown. PN comprises 28% of our cases, a percentage similar to that found for non-typable strains (9/28) or for groups $0_6$, $0_{18}$, $0_{22}$, (7/24). But a significant difference is suggested for the rough strain group observed in four out of six cases of PN.

**Conclusion**

Production of IgG antibodies is a specific response in PN, and their study allows a better evaluation of the renal involvement in UTI. In a small percentage of lower UTI, antibodies are detected either in urine or in serum. For the nephrologist, these so-called 'false positives' are the cases to be followed carefully, in order to know if they progress to PN.

**References**

1 Holmgren, J, and Smith JW (1975) *Prog. Allergy*, 18, 289
Open Discussion

KERR (Newcastle) Firstly, we have had considerable difficulty in getting reproducible results with this test and I would like to know if you have done a double-blind observer-error study on the test or whether the specimens were examined with any knowledge of the suspected clinical diagnosis.

FRIES The technician who performed the tests was not aware of the clinical condition of the patients, so this was a blind study.

KERR Secondly, if I understood your figures correctly, 90% of all the human samples in chronic pyelonephritis were positive. This is surprising since it suggests that almost every urinary infection in a woman with chronic pyelonephritis is an upper urinary tract infection which does not seem to accord with the clinical impression that patients with chronic pyelonephritis often develop lower UTI. Were these symptomatic or asymptomatic infections you were studying in your chronic pyelonephritis patients?

FRIES The difficulty lies essentially in that the urinary tract may have another infection. For instance a woman with proteus pyelonephritis can be infected by another strain. But in our homogeneous group we did not find this difficulty.

PARSONS (London) You did not tell us the time relationship between the actual acute infection and when you found your antibodies in serum and urine. Does it matter when you do the test?

FRIES All the patients with acute pyelonephritis were hospitalised. This means that the tests were done during the acute phase of their disease.

PARSONS Did therapy make any difference to the antibody titre?

FRIES Very difficult to answer that.