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Secretory immunoglobulin A and inhibitory activity of bacterial adherence to epithelial cells in urine from patients with urinary tract infections

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Summary. To assess the role of local immune response against bacterial invasion of the urinary tract we studied 168 patients with bacteriuria. Urinary secretory immunoglobulins A (sIgA) were measured using radial immunodiffusion or enzyme-linked immunosorbent assay (ELISA). In particular, ELISA is a very suitable assay for measuring the low levels of sIgA in urine. Furthermore, we used a quantitative in vitro adherence assay to investigate the attachment of *Escherichia coli* to human uroepithelial cells after incubation in urine from patients with urinary tract infection. Urine from patients with ileocystoplasty was significantly more potent in inhibiting bacterial adherence than was urine from other groups of patients with urinary tract infection. The presence of high urinary sIgA may help explain the increased antiadherence activity of urine in patients with ileocystoplasty. Mean urinary sIgA in patients with upper urinary tract infection was higher than in patients with uncomplicated infection in the lower urinary tract. Alterations in mucosal immune functions may account for the propensity toward bacterial colonization in women prone to uncomplicated urinary tract infection.

Key words: Secretory immunoglobulin A – Bacterial adhesion – Urinary tract infection – Ileocystoplasty

In some patients, susceptibility to urinary infections is due to the presence of obstruction and anatomic abnormalities; in other patients with non-obstructive urinary tract infection, microbial colonization of the urinary tract depends on the ability of bacteria to adhere to the mucosal uroepithelial surfaces. Bacterial adhesion may be related to multiple host-parasite factors, such as the virulence of bacteria, the antiadherence properties of mucosal surface, the presence of receptors on uroepithelial cells that bind specifically uropathogenic bacteria, and the immune response.

Over the last few years increasing emphasis has been placed on the importance of the role of local mucosal immunity in resistance against urinary tract infections [8, 16]. In vitro and in vivo studies have demonstrated that bacterial adhesion to uroepithelial cells can be inhibited immunologically [5, 10–15]. Antibodies may react with the bacterial ligands (fimbriae and pili), thus blocking their attachment to specific epithelial cell receptors.

The absence of antibodies in the urine or in the vaginal and prostatic fluids may account for the propensity toward colonization with potential pathogens. In particular, immunoglobulin A has been demonstrated to agglutinate bacteria, facilitating their clearance by the washout mechanism.

Secretory immunoglobulin A (sIgA) consists of two immunoglobulin A molecules and a ligand termed "secretory piece"; the immunoglobulin A portions are synthesized locally by plasma cells in the lamina propria, while secretory piece is synthetized in epithelial cells and then attached to immunoglobulin A in transit across the epithelium.

Immunostimulation may lead to increased secretion of sIgA in the urinary tract and thus to defence against infection [4, 20]. In the present study to investigate the local immune response we measured the concentration of sIgA and the inhibitory activity of bacterial adherence in urine samples from patients with urinary tract infection.

Materials and methods

A total of 168 patients with significant bacteriuria ($> 10^5$/ml) were studied. The first group of 73 patients had an uncomplicated infection of the lower urinary tract which was diagnosed because of its frequency and the dysuria associated with normal laboratory findings. This group included 67 women and 6 men (mean age 45 ± 16 years). The second group consisted of 90 patients with infected renal stones (upper urinary tract infection). This cohort comprised 63 women and 27 men (mean age 48 ± 15 years). The third group comprised 5 patients with ileocystoplasty (2 women and 3 men; mean age 53 ± 5 years).

The investigation included plain films of the abdomen, renal ultrasound scanning and/or urography. Urine specimens were

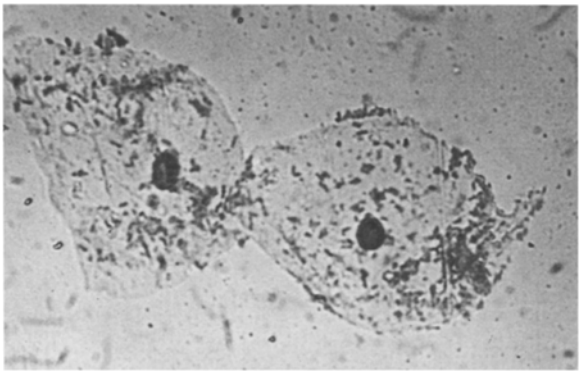

Fig. 1. *Escherichia coli* adhering to human female voided epithelial cells

Table 1. Organisms isolated from 168 patients with urinary tract infection (UTI)

Lower uncomplicated UTI	
Escherichia coli	62/73
Staphylococci	4/73
Enterococci	4/73
Proteus spp	3/73
Upper complicated UTI	
Escherichia coli	37/90
Pseudomonas spp	18/90
Proteus spp	15/90
Klebsiella spp	5/90
Enterococci	5/90
Serratia spp	4/90
Providencia spp	2/90
Enterobacter spp	2/90
Staphyloccus	1/90
Acinebacter	1/90
Ileocystoplasty	
Pseudomonas spp	3/5
Proteus spp	2/5

collected at the first morning voiding using a clean-catch technique within 48 h after the onset of symptoms prior to administration of antibiotics. In one specimen a urinary quantitative culture was performed. Two specimens were stored at −20° for determination of creatinine (colorimetric method) and sIgA (radial immunodiffusion or ELISA).

In 32 patients a fourth specimen was filtered down onto an 0.45 μm Nucleopore filter to remove cells, crystals and bacteria: the filtered urine was used for bacterial adherence test. In 81 samples urinary sIgA levels were measured by an immunodiffusion method while in the remaining 87 samples the measurement was performed by enzyme-linked immunosorbent assay (ELISA). Immunodiffusion assay was performed by single radial diffusion with monospecific antisera obtained by immunized rabbits with highly purified human IgA (LC-Partigen Behring). The urine samples had to be concentrated 50-fold using an Amicon cell to quantify small amounts of immunogloublins in urine.

In contrast the ELISA was performed in unprocessed urine (Immuno Pharmacology Research): (1) the wells of a microtiter plate were coated with antisecretory component; (2) sIgA conjugated with alkaline phosphatase was added to urine or standard samples, transferred in each well and then incubated for 1 h at 37 °C; (3) the plates were washed and dried, the substrate was added to each well, and the plates were incubated for 30 min; (4) the reaction was stopped by adding sodium hydroxide and the absorbance of the wells was measured using a microplate reader at 405 nm; (5) standard curves were made with a standard serum with sIgA. Urinary sIgA to creatinine ratio (sIgA/Cr) was calculated to compensate for the effect of diuresis. The capacity of urine to inhibit the attachment of bacteria to human epithelial cells was determined in 32 samples. Adhesion-inhibiing tests were performed according to the method described by Svanborg Edén [13].

An *E. coli* strain, isolated from a patient with a serious febrile urinary tract infection, was subcultured and kept on deep agar slants until used for a given experiment. This strain had shown both mannose-resistant and mannose-sensitive agglutination and adhesive capacity in vitro to human uroepithelial cells (kindly supplied by Dr. Savoia, Turin, Italy).

Briefly, 1 ml of a suspension of uroepithelial cells from the urine of a single healthy donor (10^5 cells per ml) was mixed with an equal volume of bacterial suspension (10^8 bacterial cells per ml).

An aliquot of filtered urine was added to test its ability to inhibit bacterial attachment to cells. At the same time a control suspension was treated with an equal volume of phosphate-buffered saline (PBS). The suspensions were incubated for 1 h at 37 °C. The epithelial cells were washed three times and filtered down onto a Nucleopore filter. The bacteria adhering to each cell were counted up to a total of 40 cells by direct light microscopy (Fig. 1). The inhibiting effect was calculated as relative adhesion, dividing the mean number of bacteria adhering to cells in the presence of urine by the mean number of bacteria adhering to cells in control suspensions.

Results

In both lower and upper urinary tract infections the most frequently identified organism was *E. coli*; *Staphylococcus saphropyticus* was the second most commonly encountered bacterium in bladder infections, and *Pseudomonas aeruginosa* was found in renal infections (Table 1).

The amount of urinary sIgA was sufficient to allow quantitation in 34 out of 81 patients by radial immunodiffusion (42%), while the sIgA concentration were detectable by ELISA in all but one urinary sample (98%). The highest levels of sIgA to creatinine ratio were observed in the urine of patients with ileocystoplasty. Mean urinary sIgA to creatinine ratio in patients with infection of the upper urinary tract or infected urinary tract abnormalities was higher than in patients with uncomplicated lower urinary tract infections. The statistical comparison of mean values indicated a significant difference between groups by the analysis of variance, whereas the values measured by ELISA were not significantly different (Table 2).

In 31 healthy subjects (23 women and 8 men, mean age 36 ± 7) the mean urinary sIgA to creatinine ratio measured by ELISA was 0.340 ± 0.323 mg/g. The upper limit of our normal range is 1 mg/g (mean plus two standard deviations). The mean value of bacterial relative adhesion to uroepithelial cells in the presence of urine of patients was higher than the mean value in the presence of urine of patients with ileocystoplasty or upper urinary tract infection. The difference between groups was statistically significant by the analysis of variance (Table 3). Furthermore, the bacterial relative adhesion data were analyzed for differences between the two groups by employing

Table 2. Urinary sIgA to creatinine ratio (mg/g) in patients with urinary tract infection (UTI)

	Mean	SD	Cases
Total			
Lower UTI	0.3488	0.6691	73
Upper UTI	0.5933	1.0560	90
Ileocystoplasty	1.6966	0.7098	5
Analysis of variance: significance 0.0034			
Immunodiffusion			
Lower UTI	0.0238	0.0628	36
Upper UTI	0.4531	1.3063	44
Ileocystoplasty	2.2093	0.0	1
Significance 0.0237			
ELISA			
Lower UTI	0.6650	0.8265	37
Upper UTI	0.7274	0.7330	46
Ileocystoplasty	1.5684	0.7498	4
Significance 0.09112			

Table 3. Relative adhesion to uroepithelial cells in patients with urinary tract infections (UTI)

	Mean	SD	Cases
Lower UTI	0.8786	0.1972	21
Upper UTI	0.8075	0.2093	8
Ileocystoplasty	0.5433	0.0950	3
Analysis of variance: significance 0.0305			

Student's *t*-test: there was no statistically significant difference between patients with lower and upper urinary tract infection. On the contrary, the mean value of bacterial relative adhesion was statistically higher in the ilecystoplasty group compared to both patient groups with lower urinary tract infection ($p < 0.01$) and the group with upper urinary tract infection ($p < 0.05$).

Discussion

Several methods are available for the measurement of immunoglobulins, but most are not suitable for quantifying small amounts of immunoglobulins in urine. Some authors [6, 18, 19] have measured urinary sIgA by radial immunodiffusion. This technique requires concentrating urine samples 50–100 times by ultrafiltration to detect low levels of urinary immunoglobulins, but filtration may cause an unpredictable loss of immunoglobulins. In fact, we were able to measure sIgA by radial immunodiffusion in only 40% of urinary samples. Patients with higher or complicated urinary tract infections often develop sIgA levels high enough to be measured by radial immunodiffusion. However, this method is not sensitive enough to quantitate sIgA levels in most normal subjects and in patients with uncomplicated urinary tract infections.

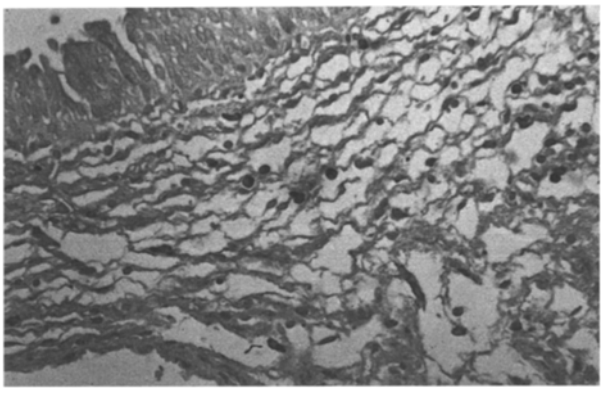

Fig. 2. Bladder tissue in lower urinary tract infection stained by perioxidase antiperoxidase staining: very few immunoglobulins A plasma cells are present

For this reason we have subsequently adopted an enzyme-linked immunosorbent assay that is more sensitive for measuring small amounts of immunoglobulins [1, 9, 17]. This technique allowed us to extend our investigation to sIgA levels even lower than the normal range.

Several patients with higher urinary tract infection developed high levels of urinary sIgA; in contrast, lower levels of urinary sIgA are detected in patients with uncomplicated infection of the lower urinary tract. However, sIgA quantitative determination by ELISA assay has not been completely useful in localizing the urinary tract infection site due to the considerable overlap between the urinary sIgA levels of those judged to have upper or lower urinary tract infections.

Our data confirm that urinary sIgA is not deficient in complicated urinary tract infections; in contrast, a low mucosal sIgA level might be a host factor that predisposes a limited group of women to have non-obstructive recurrent urinary tract infections [9].

On the other hand, lower urinary sIgA in bladder infections may be due to a reduced local immune response. This belief is enforced by the studies of Hjelm [2] in the rat with ascending urinary tract infection induced by *E. coli*. In that study infection slightly increased the number of T helpers and IgA-producing cells in the bladder submucosa without any increase in IgG or IgM producing cells, whereas in the renal tissue large amounts of T cells and immunoglobulin-producing cells were observed.

Furthermore, very few IgA plasma cells can be found beneath the bladder epithelium of women with uncomplicated urinary tract infection (unpublished data) (Fig. 2). These findings support the view that the importance of sIgA in providing local protection to lower urinary tract infections is questionable.

Unfortunately, no concomitant serum and urine immunoglobulins determination was done in our patients, so no correlation can be drawn between the urine and serum levels. However, Hopkins et al. [3] have demonstrated

the capacity of primates to mount both local and systemic immune responses against induced urinary tract infection. The results of the present study suggest that high sIgA in patients with ileocystoplasty interfere with bacterial adhesion to uroepithelial cells. In particular, sIgA produced by the intestinal mucosa may play a role in the defense against urinary tract infection in patients with ileocystoplasty [7].

Our in vitro assay utilizes a little aliquot of urine compared to the number of bacteria in the suspension. Therefore, the final concentration of immunoglobulins in the majority of test suspensions may be too low to significantly decrease bacterial adherence to voided human epithelial cells. Thus, only urine from the ileocystoplasty group that contained the greatest quantity of immunoglobulins showed a significant antiadherence effect. A more sensitive assay might show subtle differences in the antiadherence activity of urine from patients with lower and upper urinary tract infections.

Finally, the antiadherence activity in urinary tract is mediated by factors different from antibodies: the mucin layer on the bladder surface may inhibit bacterial adherence to mucosal surfaces. In conclusion, a comprehensive study of multiple host-parasite factors is needed for a better understanding of the pathogenesis of non-obstructive urinary tract infections.

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