

Does low urinary sIgA predispose to urinary tract infection?

GERD RIEDASCH, PETER HECK, ERNST RAUTERBERG, and EBERHARD RITZ

Departments of Urology, Immunology, and Internal Medicine, University of Heidelberg, Federal Republic of Germany

Does low urinary sIgA predispose to urinary tract infection? Median urinary secretory IgA (sIgA) (ELISA technique in unprocessed urine) was 1.36 mg/liter (range, 0.29 to 2.31) in healthy female controls at various times of the menstrual cycle. It was significantly lower in women with urinary tract infection (UTI) without antibody-coated bacteria. Such decrease was found both in women with acute UTI episodes (median, 0.16; range, 0.06 to 1.71) and in asymptomatic nonbacteriuric women with a history of UTI (median, 0.52; range, 0.05 to 2.13). In the latter women, sIgA in nasal secretions tended to be low, but salivary sIgA was unchanged. Urinary sIgA was elevated significantly in individuals with nephrostomy and antibody-coated bacteria (14.4 mg/liter, range, 3.6 to 20). The study showed that locally synthesized sIgA immunoglobulins were low in the urine of individuals with recurrent UTI independent of the presence or absence of bacteriuria at the time of the study. UTI per se did not interfere with sIgA secretion as shown by high sIgA in patients with upper UTI. Low urinary sIgA may represent one factor predisposing to recurrent UTI.

Des sIgA urinaires basses prédisposent-elles à l'infection urinaire? La médiane des sIgA urinaires (technique ELISA sur des urines non traitées) était de 1,36 mg/litre (extrêmes 0,29 à 2,31) chez des femmes contrôles normales à différentes périodes du cycle menstruel. Elle était significativement plus basse chez les femmes ayant une infection urinaire (UTI) sans bactérie recouverte d'anticorps. Cette diminution a été trouvée chez les femmes avec des épisodes d'UTI aiguë (médiane, 0,16; extrêmes 0,06 à 1,71) et chez des femmes nonbactériuriques asymptomatiques avec une histoire d'UTI (médiane, 0,52; extrêmes 0,05 à 2,13). Chez ces dernières, les sIgA des sécrétions nasales tendaient à être basses, mais les sIgA salivaires étaient inchangées. Les sIgA urinaires étaient significativement élevées chez des sujets ayant une néphrostomie et des bactéries recouvertes d'anticorps (14,4 mg/litre; extrêmes, 3,6 à 20). Cette étude montre que les immunoglobulines sIgA synthétisées localement sont basses dans les urines de sujets ayant des UTI récidivantes, indépendamment de la présence ou de l'absence de bactériurie au moment de l'étude. L'UTI en soi n'interfère pas la sécrétion de sIgA comme le montre les sIgA élevées chez des malades avec des UTI du tractus supérieur. Les sIgA urinaires basses pourraient constituer un facteur prédisposant à des UTI récidivantes.

Bacterial adhesion to epithelial cells is a crucial event in the initiation of urinary tract infection. In individuals with recurrent urinary tract infections, an imbalance between factors which promote bacterial adhesion and factors which interfere with bacterial adhesion are thought to condition repeated episodes of ascending infections of the urinary tract (UTI).

Secretory IgA (sIgA), an immunoglobulin synthesized locally in mucosal surfaces, plays a pivotal role in preventing mucosal colonization by pathogenic bacteria [1]. This has been well studied in the intestine [2]. A similar role has been postulated for the urinary tract, although its protective effect in this location is less well documented.

Measurement of sIgA in biological fluids is beset with many methodological problems. According to Åkerlund et al [3], consistent but variable losses of sIgA occur when urine is concentrated or stored. Immunodiffusion methods are too insensitive to permit accurate measurements of urinary sIgA. Recently, the introduction of enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) techniques has permitted the measurement of sIgA in unprocessed native urine [3-5].

In this study, sIgA concentrations were examined with the ELISA technique in urine, saliva, and nasal secretions of women with urinary tract infection.

Methods

Probands. As controls, 46 healthy women (median age, 26 years; range 18 to 40) without a history of urinary tract infection were studied in various phases of the menstrual cycle. In all female controls sIgA was measured in morning urine specimens. In 37 of the 46 controls saliva was examined and in 20 of the controls nasal washings were also examined. In addition, five women (20 to 35 years) measured basal temperature and were studied on days 4, 9, 16, and 23 of their menstrual cycle. One of the probands used oral contraceptives and the others took no hormonal medication. Furthermore, six healthy men, aged 25 to 30 years, were examined.

The following patient groups were studied: (1) 13 bacteriuric women (median age, 27 years; range, 18 to 35) with a history of recurrent (\geq three episodes) urinary tract infection (UTI) immediately after admission for dysuria within 48 hr after the onset of symptoms prior to administration of antibiotics; six women had subvesical narrowing, and seven had no urethral abnormalities; (2) nine women (median age, 26 years; range 17 to 34) with a history of recurrent UTI who had neither symptoms nor bacteriuria at the time of the study. In seven of these nine women sIgA in saliva and nasal secretions were also measured; (3) six women (median age, 22; range, 17 to 24) without a history of recurrent UTI who presented with acute dysuria and frequency without fever or flank pain; all had significant bacteriuria (\geq 100,000/ml) and had received no antibiotics; (4) ten individuals (seven women and three men,

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Table 1. Urinary sIgA in women with urinary tract infection (UTI)

Groups	N	mg sIgA/liter	mg sIgA/g creatinine
Healthy controls	46	1.36 (0.29 to 2.31)	1.15 (0.40 to 1.93)
Acute episode of UTI	13	0.16 ^a (0.06 to 1.71)	0.62 ^a (0.06 to 1.17)
History of UTI, asymptomatic without bacteriuria	9	0.52 (0.05 to 2.13) ^a	0.61 (0.04 to 0.92) ^b
No history of UTI, symptomatic episode with bacteriuria	6	0.29 ^a (0.14 to 0.91)	0.34 ^b (0.10 to 1.32)
Nephrostomy	10	14.4 ^a (3.6 to 20)	16.7 ^a (2.35 to 42.5)

Abbreviation: N, number of patients or controls studied.

Significant difference between probands and controls:

^a $P < 0.001$

^b $P < 0.05$

aged 20 to 70 years) with tube nephrostomy for greater than or equal to 10 days and with bacteriuria and carcinoma of the bladder ($N = 5$) or ureteral stones ($N = 5$).

Collection of urine. In both normal probands and outpatients, morning urine was obtained using the midstream technique. In one specimen, urine bacteriology was examined with the dip slide technique (Uricult®). Another specimen was deep frozen (-20°C) for urinary creatinine (Technicon Autoanalyser). The rest was used for immediate determination of sIgA.

Collection of saliva. Salivary flow was first stimulated by pipetting 1 ml of lemon juice into the mouth of the proband. After 1 min the proband was asked to spit out all saliva. Subsequently, a tube (PVC, ID 2 cm) with a bevelled end was pressed tightly underneath the opening of the parotid duct. Another milliliter of lemon juice was administered to the tongue, and parotid saliva was collected. The specimens of saliva were used for direct determination of sIgA (ELISA) and sodium (flame photometry).

Collection of nasal secretions. The probands were seated with their necks hyperextended while 5 ml isotonic 37°C saline were pipetted into each nostril. The probands were then asked to tilt their heads forward and blow through their noses. The fluid, approximately 7 ml, was caught in a plastic container and centrifuged (10 min, 3000 rpm). The supernatant was used for direct determination of sIgA.

Measurement of sIgA. Reagents: Antiserum against human alpha chains (2.9 g ab/liter) prepared from goat was obtained from Hyland Laboratories, Costa Mesa, California. As the sIgA standard, cooled human maternal milk was used which was kindly provided by Dr. L. A. Hanson (Göteborg, Sweden). The concentration was 1.2 g sIgA/liter. Rabbit anti-secretory component (SC) was obtained from Dakopatts AS, Copenhagen, Denmark, and was conjugated with alkaline phosphatase with a specific activity of 990 U/mg protein (Sigma Chemical Company, St. Louis, Missouri). Nitrophenyl-phosphate (Sigma Chemical Company) was used as a substrate.

sIgA was measured according to Åkerlund et al [3]. Plastic tubes (55 × 11 mm, Heger Plastiks AB, Stallarholmen, Sweden) were coated with 0.9 ml of a serum IgA pool (13 mg/ml) diluted

Table 2. sIgA of saliva and nasal secretions in asymptomatic women with recurrent urinary tract infection (UTI)

	Saliva		Nasal secretion
	mg sIgA/liter	mg sIgA/mole Na	mg sIgA/liter
Control ($N = 37$)	51.5 (11.8 to 200)	1.33 (0.07 to 11.8)	29.3 (5.0 to 133)
UTI ($N = 7$)	52.8 (36.5 to 91.5)	1.26 (0.18 to 5.53)	6.25 ^a (3.3 to 7.6)

^a Value reflects a significant difference between probands and controls ($P < 0.01$).

1:1000 in PBS (pH 7.2) containing 3 mmoles NaN_3 . The tubes containing the serum IgA were rotated with a slanted rotor driven by an electromotor at 37°C for 3 hr. Subsequently, glutaraldehyde (0.2%) was added to a final concentration of 0.02% (to cross-link the adsorbed IgA molecules) followed by incubation of the tubes for an additional hour. The coated tubes were stored at 4°C overnight. After the tubes had been rinsed three times with PBS (pH 7.2), 0.5 ml antihuman IgA (Hyland Laboratories) was added in a dilution of 1:1000 in PBS containing 0.05% Tween 20. The tubes were incubated at 37°C using a motor driven rotor. They were subsequently kept overnight at $+4^{\circ}\text{C}$. The coated tubes were then again rinsed three times with PBS Tween. The samples to be quantitated were diluted 1:1 to 1:100 in PBS Tween and analyzed in duplicate.

The urine and standard samples were incubated in the coated tubes for 5 hr at room temperature using a roller drum. The tubes were then rinsed three times with PBS Tween. The rabbit anti-SC antiserum conjugated with alkaline phosphatase was added to the rinsed tubes in a final concentration of 0.3 mg enzyme/liter in PBS Tween and was allowed to react with sIgA attached to the tubes via the anti-alpha-antiserum during incubation overnight on the roller drum. sIgA was finally estimated by measuring the enzyme activity remaining after rinsing. The substrate para-nitrophenylphosphate in diethanolamine buffer, 1 g/liter (1.0 M, pH 9.8 with 0.5 mmole MgCl_2) was added. After 60 to 100 min, the reaction was stopped by the addition of 50 μl 3 M NaOH. OD was measured in a Gilford flowthrough photometer at 405 nm.

From each urine specimen, a 1:1, 1:5, 1:10, 1:20, and 1:40 dilution was made. Standard curves were made with the above sIgA standard using the 1:1000 to 1:120,000 dilution. The urinary sIgA concentration was calculated using the linear part of the dilution curve of the standard. In recovery experiments, 95 to 105% of added standard was recovered.

The standard curve was linear down to concentrations of 0.03 mg/liter (detection threshold). Specificity of the procedure was shown by adding immunoglobulin preparations (Behring Company, Marburg, West Germany) to the assay; IgG (0.1 to 10 mg/liter) and IgA (0.1 to 10 mg/liter) did not change urinary sIgA measurements by more than ± 3 to 5%. In ten replicate measurements of three urine samples, CV was 7.4%; duplicate measurements for the values shown in Tables 1 and 2 differed by no more than 12%. Values exceeding such a difference were discarded.

Statistical procedures. All values are given as median and range. Statistical analysis was performed with the nonparametric Mann Whitney test.

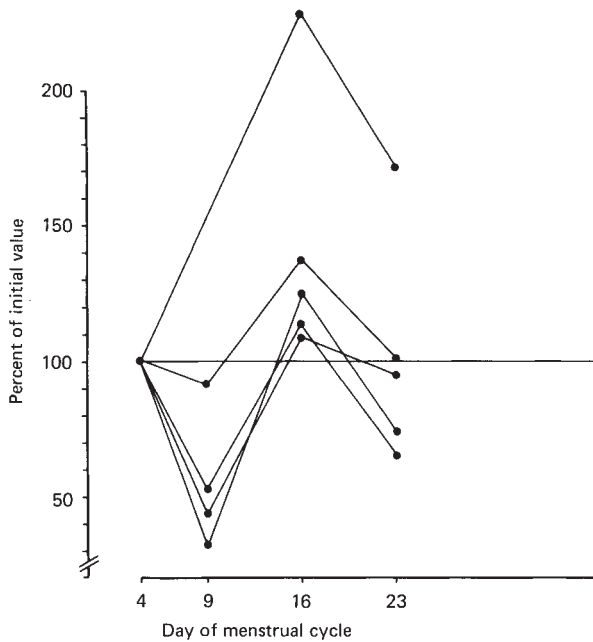


Fig. 1. Urinary sIgA concentration and menstrual cycle. Urinary sIgA was measured at days 4, 9, 16, and 23 of the menstrual cycle. sIgA concentration (mg/liter) on day 4 was taken as 100%.

Results

Urinary sIgA in control individuals. The urinary sIgA concentration in 46 healthy women is given in Table 1. In six healthy male volunteers sIgA was in a comparable range (1.6 mg/liter, 1.25 to 2.14) as in females. Five women were studied at various phases of the menstrual cycle. As shown in Figure 1, peak concentrations of urinary sIgA were seen on the day after ovulation.

Urinary sIgA in women with UTI. As shown in Table 1, significantly lower urinary sIgA were found in women with an acute episode of UTI. The difference between controls and individuals with UTI is also evident from the histogram given in Figure 2. Similarly low sIgA were found in UTI women with ($N = 6$; median, sIgA 0.245 mg/liter; range, 0.13 to 1.17) and without urethral narrowing ($N = 7$; median sIgA 0.115; range, 0.06 to 1.71).

Low urinary sIgA were also found, however, in asymptomatic women with a history of UTI without bacteriuria at the time of the study and in women with an acute episode of bacteriuria without a history of UTI. Increased urinary sIgA were observed in individuals with tube nephrostomy. Antibody-coated bacteria, studied as described previously [6], were absent in all bacteriuric individuals without nephrostomy and present in all with nephrostomy.

sIgA in saliva and nasal secretions. The sIgA concentration in saliva and nasal secretion of asymptomatic women with a history of UTI is given in Table 2. There was no difference of salivary sIgA concentration irrespective of the frame of reference (per liter; per mmole Na; per minute). In contrast, low sIgA were found in nasal secretions of women with recurrent UTI. With the exception of one control proband, there was no overlap between values in controls and UTI patients.

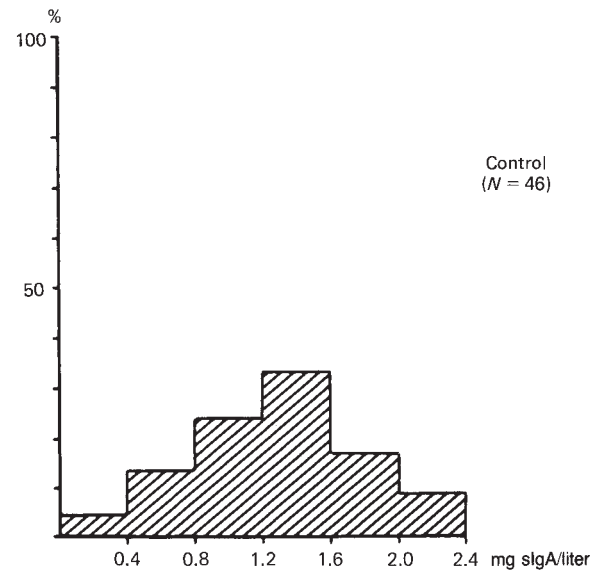
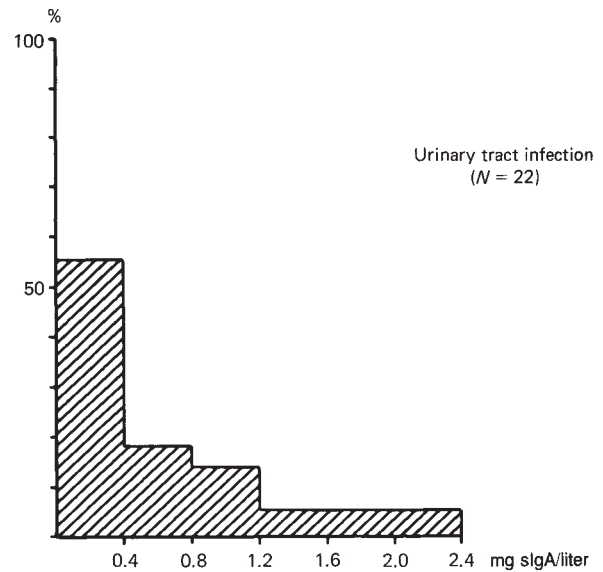


Fig. 2. Frequency distribution of urinary sIgA concentration in women with urinary tract infection and healthy control women. In the upper panel, 22 women with a history of UTI, either bacteriuric ($N = 13$) or nonbacteriuric ($N = 9$) at the time of the study, were examined. Whereas the distribution is approximately Gaussian in control women, it is markedly skewed in women with a history of UTI.

Discussion

This study shows low urinary sIgA concentrations in women with recurrent UTI irrespective of whether they were bacteriuric or not at the time of the study. Women with an acute attack of UTI without a history of UTI also had low urinary sIgA. Given their young age, such probands may represent women susceptible to recurrent UTI who had experienced their first attack, but this assumption remains conjectural.

The urinary sIgA concentrations of unprocessed urine found in the present study agree with values in recent studies using similar techniques [3] or radioimmunoassay [4]. Lower normal values were previously reported by Kaufmann, Katz, and

McIntosh [7] and Uehling and Steihm [8] and Uehling [9], but in their studies, 24-hr urine was stored, concentrated, and examined with radial immunodiffusion. As discussed by Åkerlund et al [3], the storing and processing of urine may lead to a variable loss of sIgA. Several artefacts were considered and excluded by appropriate procedures. First, urinary sIgA in women depend on the phase of the cycle. The change of sIgA concentration with the menstrual cycle was much larger than the error of replicate measurements which did not exceed 10%. However, low urinary sIgA in recurrent UTI cannot be due to the clustering of UTI episodes at the time of menstruation, since the episodes were distributed randomly throughout the menstrual cycle (data not given). Artefactual lowering of urinary sIgA by water diuresis is excluded by the finding of low urinary sIgA when factored for urinary creatinine. It is unlikely that other proteins interfere with the assay in women with UTI, since LC (low concentration) urinary diffusion plates for IgG and transferrin (Behring Company) were examined routinely and these consistently failed to show the presence of IgG or transferrin except in individuals with nephrostomy. Finally, consumption of sIgA by urinary bacteria, for example, by proteolytic cleavage [10], is unlikely. Seeding of urine with a reference strain of *Escherichia coli* (100,000/ml) and incubation for 24 hr at 37°C failed to decrease sIgA by more than 15%, while the sIgA concentration of noninfected urine under the same conditions changed by no more than $\pm 3\%$.

The finding of lower urinary sIgA supplements previous findings of Tuttle, Sarvas, and Koistinen [11] who observed lower vaginal immunoglobulin A in girls with recurrent UTI when compared to an age-matched group of controls who had never experienced bacteriuria. Kurdydyk et al [12] found no difference of vaginal IgA in women with recurrent UTI when compared to controls, but this must be interpreted with caution, since after menarche the composition of vaginal secretion depends upon the phase of the menstrual cycle [13]. More recently, Stamey et al [14] showed that IgA and IgG concentrations are reduced in susceptible and bacteriuric women with introital carriage of *Enterobacteriaceae*. Specifically, the authors showed antibody coating of *Enterobacteriaceae* with cervicovaginal antibody in a lower proportion of women susceptible to urinary tract infections than in control subjects resistant to urinary tract infection.

Urinary sIgA concentrations, as measured by immunodiffusion, were not different between control women and women with recurrent UTI in the study of Uehling [9], but this technique of sIgA measurement does not reliably exclude sIgA loss as discussed by Åkerlund et al [3].

The presence of low urinary sIgA could be the cause or the consequence of urinary tract infection.

It is unlikely that luminal infections (negative antibody coating) could interfere with local sIgA synthesis. First, Hand et al [15] found no change of sIgA synthesis in experimental bladder infections. Second, sIgA levels were low in acutely symptomatic urinary tract infection without antibody-coated bacteria but consistently elevated in patients with nephrostomy and antibody-coated bacteria. This agrees with previous observations of Uehling and Steihm [8] who found higher urinary sIgA in UTI of children with severe anatomic derangements of urinary tracts. Our experimental studies show that the mucosa of the urinary tract can mount a local immune response if the damaged

lamina propria is invaded by bacteria [16]. It is therefore unlikely that low urinary sIgA in UTI are an expression of damage to the uroepithelium.

The alternate hypothesis would be that low urinary sIgA concentrations are causally related to UTI. The role of sIgA in the genesis of UTI in our patients is difficult to evaluate because the antigenic specificity of sIgA was not examined. However, it is known from previous studies of Svanburg-Edén [17] that antibodies of the sIgA class may prevent adhesion of *E. coli* to human urinary tract epithelial cells. Rossen et al [18] showed that low sIgA may predispose to mucosal infection. He found that nasal antibody activity in response to a challenge with influenza virus tended to be detected earlier, with symptoms developing less frequently, in those individuals who had higher baseline concentrations of IgA in nasal wash specimens. Since low urinary sIgA were also found in patients with a history of UTI but without demonstrable bacteriuria at the time of the study, it is conceivable that low sIgA may predispose to colonization of the urinary tract mucosa by pathogenic bacteria.

In this context it is intriguing that in women with recurrent UTI sIgA in nasal secretions, but not in saliva, was low in parallel with low urinary sIgA. Although statistically significant, this result must be interpreted with caution because the sample size was small. The finding would be consistent, however, with the hypothesis that low urinary sIgA is a reflection of a more general mucosal defect. This hypothesis requires confirmation by further studies involving more patients and additional mucosal secretions.

Acknowledgments

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Reprint requests to Prof. Dr. E. Ritz, Sektion Nephrologie, Klinikum der Universität Heidelberg, Bergheimer Straße 58 a, D-6900 Heidelberg 1, Federal Republic of Germany

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