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Immunohistochemical demonstration of secretory IgA in human urothelium

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Summary. The mucosal surface of the human urothelium represents a very large exposure area to exogenous agents, including potentially harmful microorganisms. Male human urothelium was treated for the immunohistochemical demonstration of secretory IgA (sIgA) in order to verify its own possible antimicrobial properties. An intense immunoreactivity for sIgA was observed in the apical cells of the urethral and vesical epithelia. The ureteric epithelium, at the luminal surface, showed discontinuous areas of less dense or completely absent reaction product. A less intense immunoreactivity was observed in the pelvic apical epithelial cells. The results suggest that sIgA play a prominent role in the local defence mechanisms of the lower urinary tract against ascending infections, whereas in the upper urinary tract the immuno-specific local defences seem reduced.

Key words: Immunohistochemistry, sIgA, Urothelium, Human

Introduction

During states of good health, the mucosal surface of the urinary tract is normally sterile. Two kinds of defensive mechanisms account for this: the dynamics of urine flow, as non specific defence; and the postulated antibacterial activity of urothelium, as specific defence (Asscher, 1980; Tomasi and Plaut, 1985).

In patients with urinary tract infections, the urine contains increased amounts of immunoglobulins of the IgG, IgM and IgA class. Whereas locally produced IgG and IgM antibodies may not be of significance in the defence of urinary tract, local production of secretory IgA (sIgA) does appear to be important (Bienenstock and Tomasi, 1968; Kaufman et al., 1970; Asscher, 1980).

Therefore, sIgA in mucosal secretions are predominant among the specific immune defences (Abraham and Beachey, 1985). Secretory IgA antibodies are ideally suited to protect the urinary tract against ascending infections. Several studies indicate that sIgA may interfere with the process of specific interaction involving adhesins on the microbial surface complementary to host surface receptors (Liljemark et al., 1979; Magnusson et al., 1979; Hanson et al., 1983; Abraham and Beachey, 1985; Kilian et al., 1988).

Because of the importance of the sIgA role in local defence mechanisms, and taking into consideration the fact that in urinary tract infections the initial pathogenic event is the attachment of bacterial to the host mucosa, we carried out an immunohistochemical study for IgA-secreting epithelial cells, to ascertain the antimicrobial properties of the human urothelium.

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Materials and methods

Biopsy specimens from male urinary bladder and urethra (prostatic, membranous and spongy portions) were obtained from ten patients, aged 40-70 years, undergoing cystectomy and urethrectomy for bladder carcinoma. Our observations were carried out only on five normal biopsies as determined by histological examination.

Ureters and renal pelvis specimens, normal at histological examination, were obtained from five patients, aged 42-57 years, undergoing nephrectomy for kidney carcinoma.

None of the patients had undergone hormonal or radiotherapeutic treatment and all were immunologically normal.

Tissue segments were fixed and processed for paraffin embedding. Microtome sections (6-7 μm) were treated for immunohistochemical demonstration of sIgA using the ABC method: they were rehydrated in PBS, pretreated with 0.1% trypsin (Sigma, St. Louis, MO) in PBS at 37 ºC for 20 min to enhance the intensity of specific staining, then immersed in a solution of 100% methyl alcohol and 0.3% hydrogen peroxide to inactivate endogenous peroxidase. The sections were treated for 15 min with 10% non immune rabbit serum. Rabbit polyclonal antibody to sIgA (Cappel, Durham, NC) was used as primary antisera (working dilution

1:4000) overnight at 4 ºC; biotinylated anti-rabbit IgG (Chemicon International, Temecula, CA; 1:200) was used as secondary antiserum for 30 min at room temperature. The sections were further incubated in avidin-biotin-peroxidase complex (Biomeda, Milano, Italy; 1:250) for 30 min at room temperature, reacted with 3,3'-diaminobenzidine (Sigma, St. Louis, MO), and then counterstained with haematoxylin. The sections were thoroughly rinsed in PBS between each step.

Furthermore, adjacent sections were incubated using rabbit anti-human IgA specific for alpha-chains, F(ab^1)2 fragment, (Dako, Glostrup, Denmark; 1:4000) as primary antiserum, for the concurrent demonstration of IgA class immunoglobulins.

In the control incubations the specificity of the sIgA and IgA specific for alpha-chains antisera was tested in adjacent sections replacing the primary antibodies with non-immune rabbit serum at the same dilution.

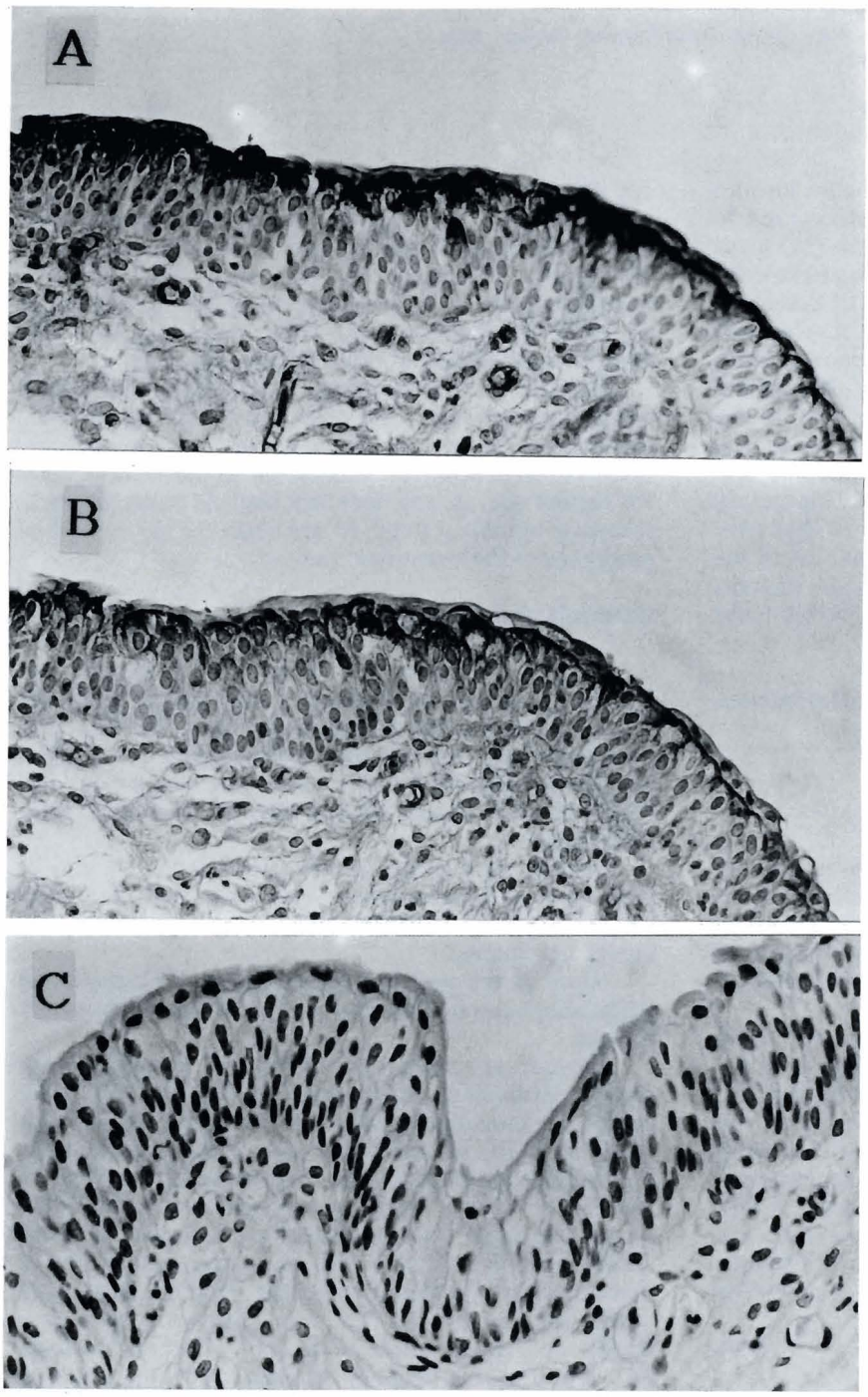

Fig. 1. Male human urinary bladder. **A.** Anti-human secretory IgA. **B.** Anti-human IgA specific for alpha-chains. **C.** Control section. A,B: an intense immunoreactivity is observed in the apical cytoplasm of the epithelial superficial cells. C: Control section appears unstained. A, B, x 225; C, x 300

Results

Immunohistochemical staining for sIgA appeared as granular cytoplasmic deposits in the superficial cells along the urothelium.

The immunoreactivity for sIgA and IgA specific for alpha chains was observed in the cytoplasm of vesical epithelial superficial cells (Fig. 1A,B). The same immunoreactivity was observed in the cytoplasm and at the luminal surface of the apical cells in all segments of the urethral epithelium (Fig. 2A).

A different pattern of immunoreaction was seen in the ureteric epithelium: the same section showed discontinuous areas of less dense or completely absent reaction product along the luminal surface (Fig. 3A,B).

A less intense immunoreactivity for sIgA and IgA specific for alpha-chains was observed in the cytoplasm of the pelvic luminal epithelial cells (Fig. 4A,B).

Immunostaining in the control sections was completely abolished (Figs. 1C, 2B, 3C).

Discussion

There are two routes by which bacteria can reach the kidneys: haematogenous and ascending infections. Ascending infections from the lower urinary tract are commonly considered the most important route (Asscher, 1980; Cotran et al., 1989). The first step in the pathogenesis of these infections appears to be the colonization of the distal urethra; from here the organisms must gain access to the bladder against the urine flow.

Our findings suggest that urethral and bladder epithelium secreting sIgA act as barrier in opposition to

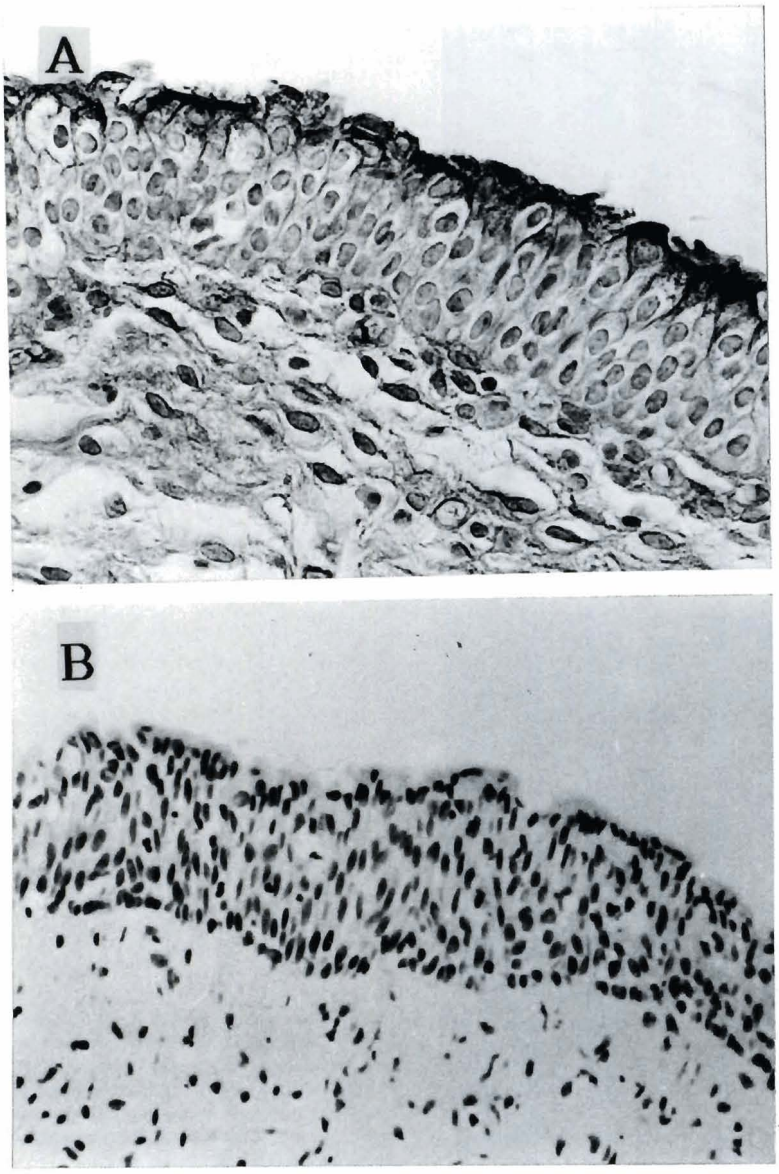

Fig. 2. Male human urethra. **A.** Anti human secretory IgA. **B.** Control section. A: Marked immunoreactivity is localized in the cytoplasm of apical cells. B: Control section appears unreactive. A, x 360; B, x 210

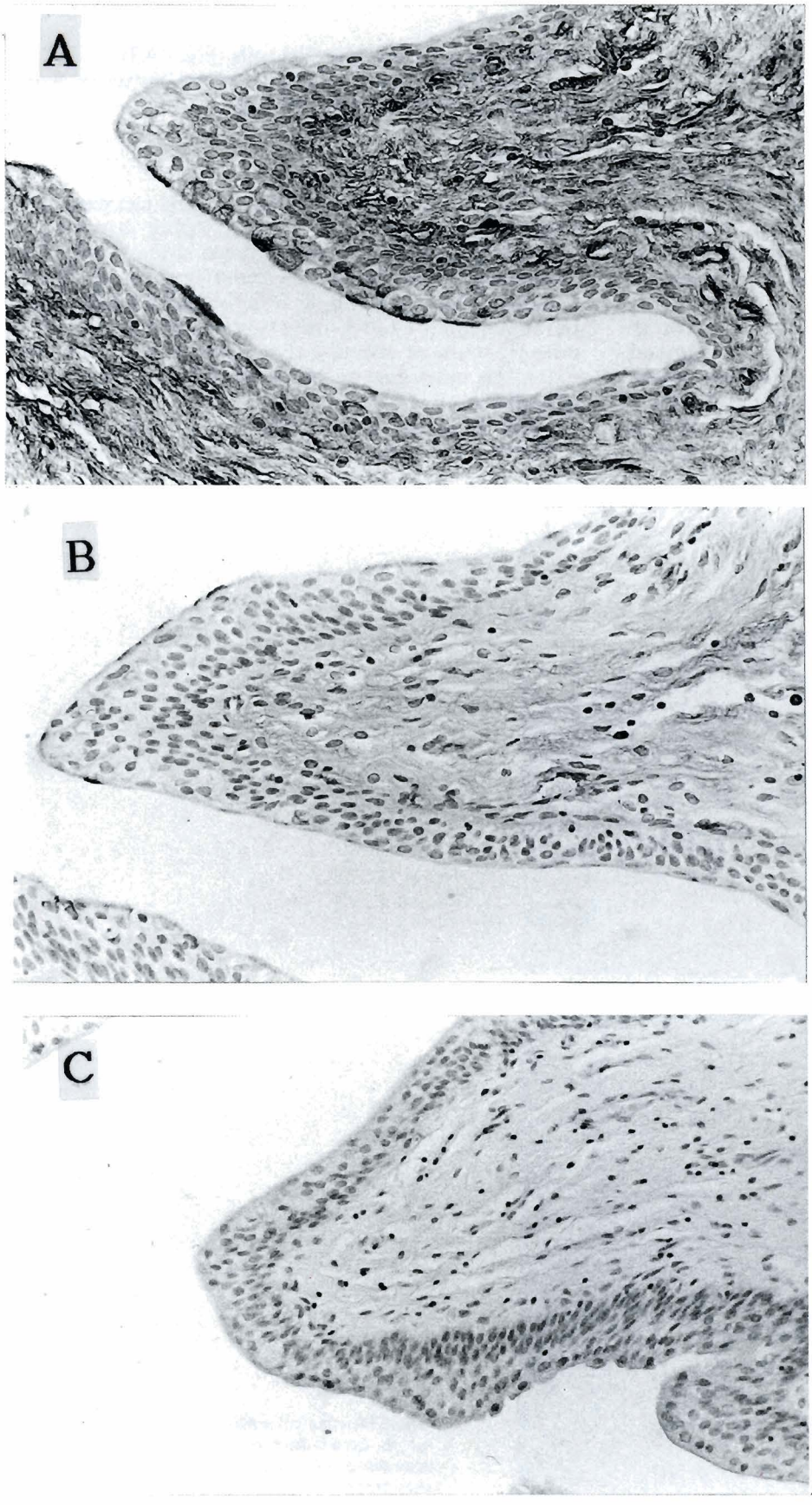

Fig. 3. Male human ureter. **A.** Anti-human secretory IgA. **B.** Anti-human IgA specific for alpha-chains. **C.** Control section. A, B: discontinuous areas of less dense or completely absent immunoreactivity are observed. C: Control section appears unstained. A, B, x 210; C, x 165

pathogenic bacteria. The mucosal immune system constitutes the first line of defence against potentially harmful substances and microorganisms (Bienenstock and Tomasi, 1968; Doe, 1989; Gomez et al., 1993). The central role of IgA in the mucosal immune response is one of the distinguishing features of the mucosal immune system. This is based on the fact that IgA has a number of properties that allow it to function more efficiently than other immunoglobulins in the mucosal environment (Strober and James, 1991). The ability of sIgA antibodies to inhibit mucosal colonization of humans and animals has been demonstrated in vivo with several species of microorganisms (Abraham and Beachey, 1985; Kilian et al., 1988). Investigations of the mechanisms underlying this ability have confirmed that sIgA antibodies exert a direct inhibitory effect on the adherence of microorganisms to host mucosal epithelial cells (Liljemark et al., 1979; Reinholdt and Kilian, 1987; Kilian et al., 1988). Although the anti-adherence effect, demonstrable in vitro, is usually relatively modest, the effect of sIgA in vivo is amplified by numerous other factors of nonimmune origin, such as the mucus coat, the continuos desquamation of the surface epithelium, and the intense competition between members of the mucosal flora (Shedlofsky and Freter, 1974; Abraham and Beachey, 1985; Kilian et al., 1988). In addition to its lubricating and transporting function, mucus secretion contains soluble compound that bind, in a competitive way, to bacterial adhesins (Abraham and Beachey, 1985). Parsons et al. (1979) demonstrated the importance of the mucus coat in reducing bacterial attachment to the surface of bladder epithelial cells. In a recent study we histochemically demonstrated the presence of acid glycoproteic material in the luminal

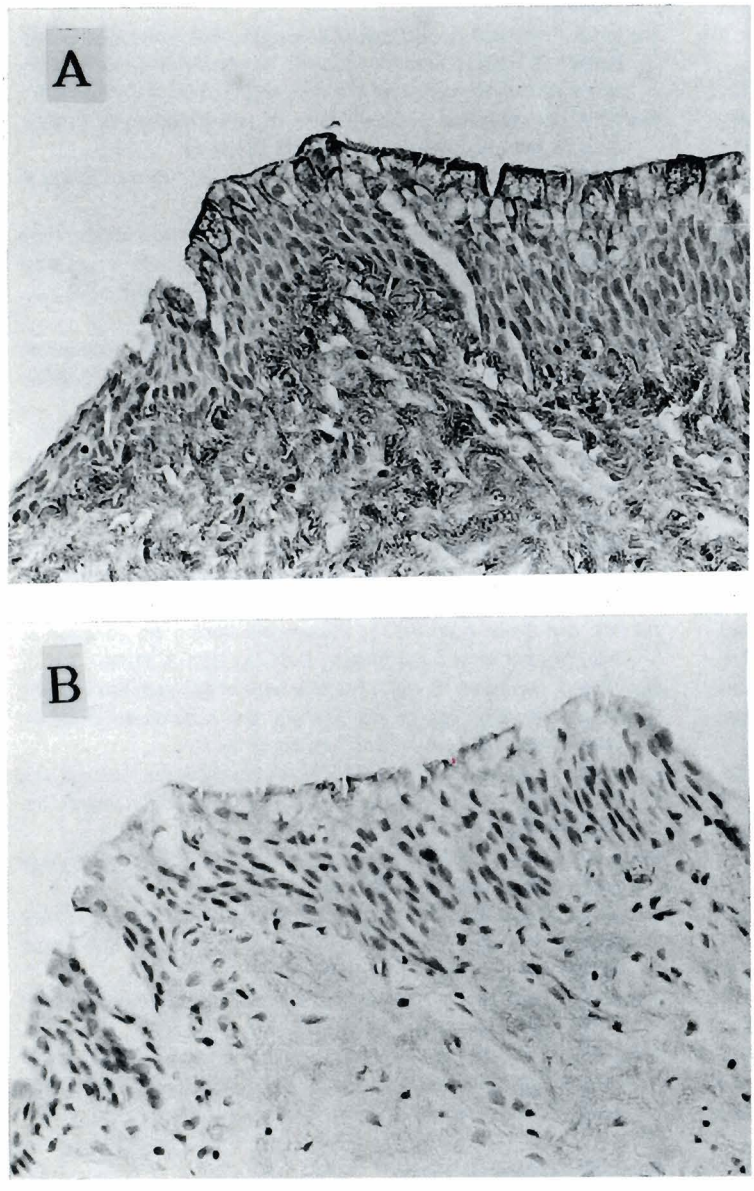

Fig. 4. Male human pelvis. **A.** Anti-human secretory IgA. **B**. Anti-human IgA specific for alpha-chains. A, B: a weak immunoreactivity is observed in the apical cytoplasm of the luminal epithelial cells. x 235

surface of the human urethral epithelium, that could prevent bacteria from migrating to the upper urinary tracts (Sirigu et al., 1993).

The specific immune defences associated with the periodic voiding of urine, and the other factors mentioned above, represent, in normal conditions, an effective protection for the lower urinary tract.

Nevertheless, it is generally accepted that in man urethral instrumentations, such as catheterization and cystoscopy, are important predisposing factors in the pathogenesis of urinary tract infections. The organisms may ascend to the bladder either along the catheter lumen by air bubbles or in the fluid film at the interface between the catheter and the urethral mucosa (Asscher, 1980; Reid and Bruce, 1993).

These ascending infections could be supported by a mechanical reduction or abolition of the physiological defensive mechanisms, like the antimicrobial properties of the urethral and bladder epithelium, such as IgA secretion, and the flushing action associated with periodic voiding of urine.

Bacteria introduced into the bladder can rapidly multiplay and, from the contaminated bladder urine, ascend along the ureter to infect the renal pelvis and the kidney parenchyma. Although obstruction is an important predisposing factor in the pathogenesis of ascending infections, it is not only the incompetence of the vesicoureteral orifice that allows bacteria to ascend up the ureter into the pelvis. As a matter of fact, once established in the bladder, uropathogens can, and occasionally do, ascend into the ureters and kidneys, with and without vesicoureteral reflux (Hagen et al., 1992).

On the ureteric tissue there are receptor sites for the uropathogens, implying that ascension into the kidneys can occur via step-wise adhesion (Reid and Bruce, 1993). The liposaccharide of Gram-negative bacteria (endotoxin) is toxic to the ureter causing decreased peristalsis, facilitating bacterial ascent to the kidney (Thulesius and Araj, 1987).

The scanty secretion of sIgA from the ureteral epithelium emphasized in our results, accounts for a rapid ascent of the pathogens to the renal pelvis, the specific defences in this part of the upper urinary tract being reduced.

Although the sIgA appears uniformly distributed, the less intense immunoreactivity observed in the renal pelvis epithelium led us to think of a minor effectiveness of the specific defences. On the other hand, since mechanical defences are reduced too, it seems likely that the renal pelvis plays a small role in the local defence mechanisms. Although further investigations are required, the present state of our knowledge may suggest that the upper urinary tract does not act as an effective barrier against ascending infections.

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