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Ultrastructural visualization of human bladder mucous

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Summary. Mucous within the urinary bladder appears to play a protective role in shielding the uroepithelium against pathogens. This present study employs specific anti-mucous, antisera stabilization techniques to visualize a thin, continuous layer of mucous closely adherent to the human bladder uroepithelium, in both scanning and transmission electron microscopic analyses.

Key words: Bladder mucous – Uroepithelium – Bacterial adherence – Urinary tract infections

Numerous intrinsic host defence mechanisms in the urinary tract are currently under active investigation [12]. The layer of mucous, demonstrated to be present as a continuous blanket in the rat bladder [3] appears to be an important intrinsic factor in shielding the epithelial layer from pathogens [2, 6]. Similarly other experimental animal studies [8] and more recently human studies [10] have indicated the important protective properties of the bladder mucosal surface or glycocalyx. In addition, it has been proposed that defects in this glycoprotein layer may lead to various pathological conditions, such as recurrent urinary tract infection (UTI) [10] and interstitial cystitis [1, 7].

Previous untrastructural investigations of human bladder urothelium have shown little evidence of the layer of mucous owing to the loss or distortion of the hydrophilic mucous during the preparative procedures for electron microscopy. In this study, human bladder mucosa was stabilized using specific anti-mucous antisera and the layer of mucous evaluated by scanning and transmission electron microscopy.

Materials and methods

Anti serum to human bladder mucous

Pooled bladder mucous was collected from cadaveric renal transplant donors and recent autopsy specimens (less than 4 h from time of death) by scraping the superficial mucosal layer with a glass spatula. The mucous solution (0.4 mg protein per ml) was frozen and stored in 1 ml aliquots at −70°C. For immunization, 1.0 ml of the mucous solution was emulsified with 2.0 ml of Freund's complete adjuvant and injected subcutaneously at multiple sites on the back of New Zealand white rabbits. Booster injections were given at 2 week invervals for a period of 8 weeks by the same technique, except Freund's incomplete adjuvant was used. The animals were bled (10 ml samples) a week after the third injection and every week after in order to quantify antibody production. The antibody activity of the serum to immobilize mucous was determined using the enzyme linked Immunosorbent Assay (ELIZA). An antibody titer of 1:40,000 was demonstrated. Immunoelectrophoresis according to Grabar and Williams [5] showed 2 major precipitin arcs. Rabbits were bled on a weekly basis following the booster regime collecting 30–50 ml blood. The serum was aliquoted in sterile vials at a volume of 2 ml frozen at −20°C and stored until used.

Patients studied

10 patients (3 male; 7 female; age range: 27–64) undergoing cystoscopy for evaluation of microscopic hematuria but no history of interstitial cystitis or previous urinary tract infections had superficial bladder biopsies taken from the bladder base with a cold cup biopsy forceps.

Stabilization of mucous

The bladder mucous layer was stabilized by gently placing the fresh bladder biopsies directly into thawed serum after collection. The biopsies were incubated in the antisera at room temperature for two hours or overnight at 4°C. Following incubation, serum was aspirated off, the tissue gently rinsed in a small volume of sterile phosphate buffered saline and then processed for scanning and transmission electron microscopy.

Processing for scanning electron microscopy

Following stabilization of the mucous, the bladder biopsy specimens were fixed for 16 h at 4°C in 5% glutaraldehyde in cacodylate buffer (0.1 M). The samples were rinsed three times in the same buffer and dehydrated in increasing concentrations of tertiary butanol. Special

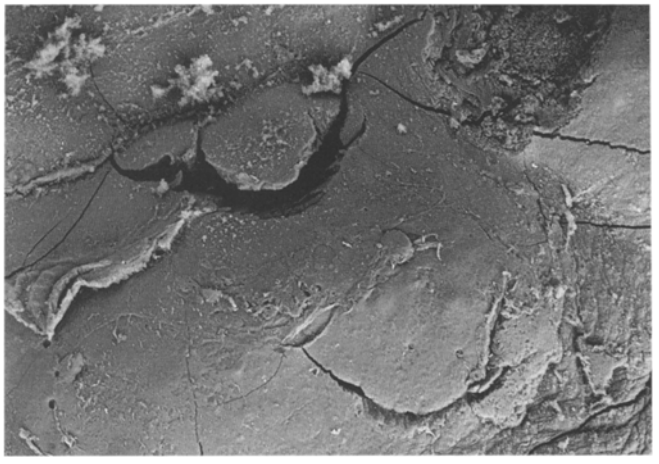

Fig. 1. Scanning electron micrograph clearly shows that the stabilized mucous layer covers the uroepithelium as a continuous blanket. (Original magnification ×57)

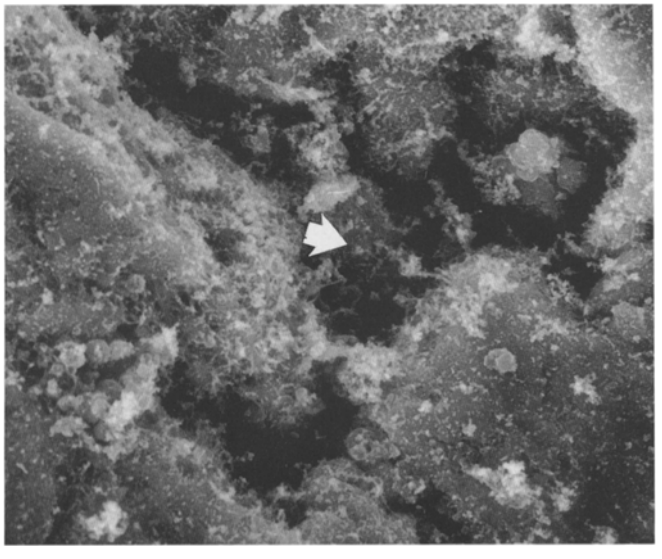

Fig. 2. This scanning electron micgrograph demonstrates the transitional epithelium (arrow) below the mucous layer in areas where the mucous has cracked and peeled back during processing. The thickness as well as the reticular and fibrillar nature of the mucous layer can be appreciated. (Original magnification ×3,000)

care was taken when changing solutions to minimize fluid sheer which might disrupt the layer of mucous. Specimens were snap frozen in liquid Freon-12 pre-cooled with liquid nitrogen, then immersed in liquid nitrogen until fully quenched and placed on a brass block pre-cooled to −196°C. All specimens were then freeze dried at −60°C for 16-24 h in an Edwards-Pearce EPD 2 tissue drier (Edwards High Vacuum Ltd. Manor Royal, Sussex, England) using phosphorous pentoxide as a vapour trap. The dried tissue specimens were orientated with the mucosal surface uppermost then mounted on aluminum stubs with double sided adhesive tape and coated with carbon then gold by vapour evaporation. The samples were examined in an ISI DS-130 research scanning electron microscope fitted with a Robinson backscattered electron detector. The accelerating voltage used was normally 30 kV.

Processing for transmission electron microscopy

Specimens were fixed in a solution of 0.1 M cacodylate buffer (pH 7.4) containing 5% glutaraldehyde and 0.15% ruthenium red for 16 h at 4°C. The samples were rinsed three times in cacodylate buffer with 0.1% ruthenium red and post-fixed in a 2:1 mixture of osmium tetroxide and 0.5% ruthenium red, both in 0.1 M cacocylate buffer (pH 7.4) for 2 h. All fixatives were freshly prepared from stock solutions prior to use. After a further three changes of 0.05% ruthenium red/cacodylate buffer wash, the specimens were dehydrated in a graded series of ethanol (30 min in each of 50, 70, 90% and absolute) followed by two 30 min changes of propylene oxide. Infiltration overnight with a 50:50 mixture of TAAB epoxy resin and propylene oxide was followed by 8 h of 75:25 resin/ propylene oxide and 24 h in 100% resin, under vacuum. The bladder tissue was embedded with the mucosal surface and polymerized under vacuum at 60°C for 16 h. Transverse sections of the bladder mucosa (1.5 μm in thickness) were stained with toluidine blue and an area of interest was isolated by trimming the block face to the toluidine blue. This enabled subsequent deeper sectioning to reveal information from several levels of the block. Thin sections (90 nm) were stained uranyl acetate and lead citrate before examination in a Philips 410LS transmission electrton microscope, operated at an accelerating voltage of 60 kV.

Results

Bladder mucosa which had been stabilized, prior to fixation for scanning electron microscopy, exhibited a continuous layer of mucous covering the urothelium (Fig. 1). In areas where the layer of mucous had cracked and peeled back due to processing artifacts, the transitional epithelium could be seen beneath the mucous (Fig. 2).

Under transmission electron microscopic examination, the bladder mucosa following stabilization with antisera and ruthenium red, revealed the mucous as an electron dense layer covering the epithelium (Fig. 3). The thickness of the layer varied, depending on how expanded the bladder tissue was. The average depth of mucous lying on unfolded epithelium was 10–20 μm, although within the folds the mucous was many times deeper.

Discussion

In this study the ultrastructural appearance of human mucous was investigated. The bladder mucous was stabilized and contrasted using specific anti-mucous antisera and ruthenium red prior to scanning and transmission electron microscopic examination. It was necessary to stabilize the mucous prior to fixation and dehydration to prevent the hydrophilic layer from colapsing [3, 9]. Ruthenium red assisted in stabilization and also increased contrast of the low electron dense glycoprotein material. Mucous was present as a continuous blanket covering the pavement-like transitional epithelium. The thickness of the layer varied, depending on the degree of stretch of the bladder mucosa, from 10–20 μm on unfolded epithelium to many times thicker within epithelial folds. This layer is relatively thin when compared to other mucous layers, such as human gastric mucous which varies in thickness from 50 to 400 μm [11].

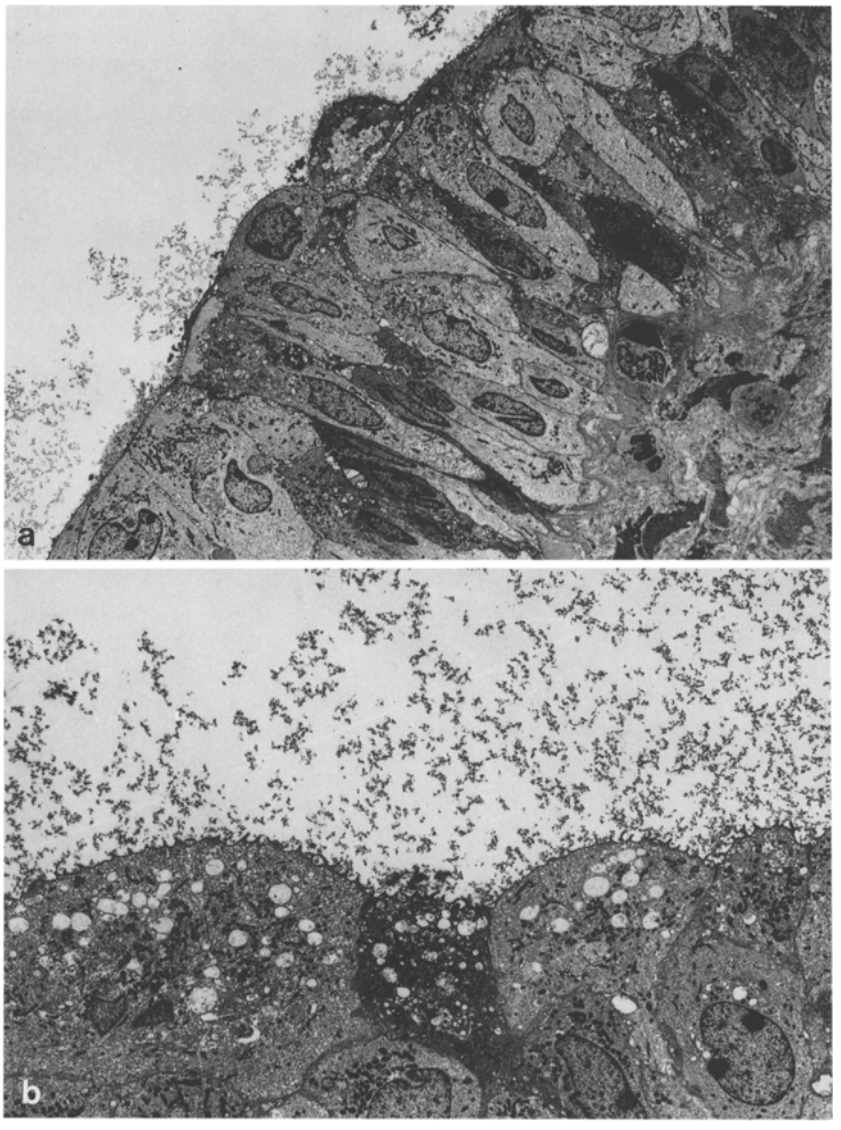

Fig. 3a and b. Transmission electron micrograph of the bladder mucosa following stabilization of the surface layer with specific antisera and ruthenium red reveals the electron dense layer covering the uroepithelium. The layer was present in all specimens but varied in thickness as demonstrated in **a** and **b.** (Original magnification: **a** ×1,570; **b** ×2,900)

The layer of bladder mucous in animals acts as an important intrinsic host defence mechanism in shielding the bladder wall from pathogens [3, 6, 8]. The ultrastructural appearance of bladder mucous in the rat bladder is similar to human mucous and it is therefore reasonable to assume that human mucous may have important similar protective properties in acting as a barrier to pathogens or toxins. A recent study investigating the potential bacterial anti-adherence nature of human bladder extracts has shown that bladder washes from patients with recurrent UTI were significantly less potent at inhibiting bacterial adherence than extracts from control patients [10]. While it may be questioned if bladder washes are a good sampler of the mucous material on the bladder surface, the results of the study imply a deficiency of the mucous layer in patients prone to UTI [12]. Similarly, a deficiency of mucous has been indicated in subjects suffering with interstial cystitis [7] although this finding is controversial [4]. Clearly it will be beneficial in investigating the pathogenesis of such diseases as UTI and interstitial cystitis, to be able to assess more accurately the morphology of diseased bladder mucosa. The method described in this paper will enable such investigations. In summary, the study has described a method of ultrastructural examination of the bladder luminal surface and has provided morphological evidence that a continuous thin layer of mucous exists within the normal human bladder mucosa.

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