Spinal Cord (2000) 38, 378–381

www.nature.com/sc

Secretory immunoglobulin A in the vesical urothelium of patients with neuropathic bladder – an immunohistochemical study

S Vaidyanathan*,[1], IW McDicken[2], BM Soni[1], G Singh[1], P Sett[1] and Nori Mat Husin[2]

[1]*Regional Spinal Injuries Centre, District General Hospital, Southport, Merseyside PR8 6PN, UK;* [2]*Department of Pathology, Royal Liverpool University Hospital, Prescot Street, Liverpool L7 8XP, UK*

Study design: A pilot study was carried out on archival material of bladder biopsies taken during 1994 and 1995 from patients with neuropathic bladder.
Objectives: To compare the pattern of immunostaining for sIgA in the urothelium of biopsies taken from neuropathic bladder with the biopsies obtained from patients with non-neuropathic bladders.
Setting: Regional Spinal Injuries Centre, Southport and Department of Pathology, Royal Liverpool University Hospital, Liverpool.
Methods: Formalin-fixed, paraffin-embedded biopsies of bladder mucosa taken from patients with neuropathic urinary bladder ($n=43$) during 1994 and 1995 were processed for immunostaining with rabbit polyclonal antibody for secretory component of IgA. Archival specimens of bladder biopsies from non-neuropathic bladder were stained as controls. All sections were stained contemporaneously.
Results: In all the control biopsies, strong immunostaining for sIgA was observed in the superficial cells of transitional epithelium. In the biopsies taken from patients with neuropathic bladder, immunostaining in the transitional epithelium was variable: strong in 14 cases; moderate in four; faint in 16; and absent in three. Immunostaining for sIgA was absent in all the five biopsies in which the urothelium had undergone squamous metaplasia. One biopsy showed intestinal metaplasia; immunostaining for sIgA was seen in the basal cells.
Conclusion: Strong immunostaining for sIgA was observed in the urothelium of all biopsies taken from non-neuropathic bladder. In contrast to this, only 18 of 37 biopsies obtained from neuropathic bladder showed strong or moderate immunostaining for sIgA in the transitional epithelium.
Spinal Cord (2000) **38,** 378–381

Keywords: immunoglobulin A; urothelium; neuropathic bladder; spinal cord injury

Introduction

Patients with neuropathic bladder are at increased risk of developing urinary infection. Incomplete bladder emptying, vesical calculus, and indwelling urinary catheters are commonly identified predisposing factors for the occurrence of cystitis in these patients. Secretory Immunoglobulin A (sIgA) plays a central role in the specific immune system; this prevents the invasion of the muscosal surface by micro-organisms. Sirigu and associates studied immunohistochemical demonstration of secretory IgA in the vesical urothelium on five normal biopsies as determined by histological examination.[1] These biopsy specimens were obtained from patients, aged 40–70 years, who were undergoing cystectomy for bladder carcinoma. An intense immunoreactivity for sIgA, and IgA specific for alpha chains was observed in the cytoplasm of the vesical epithelial superficial cells.[1]

It is possible that the structure and function of the urothelium could be altered in the patients with spinal cord injury because of damage to the nerve supply to the urinary bladder.[2] Therefore, we carried out a pilot study to investigate the pattern of immunostaining for sIgA in the vesical urothelium in archival specimens of bladder biopsies taken from (i) patients with neuropathic bladder and (ii) control group from patients with non-neuropathic bladder.

Patients and methods

The North Sefton Research Ethics Committee gave us permission to study archival specimens of bladder biopsies from patients with neuropathic bladder and from able-bodied individuals. Bladder biopsies had

*Correspondence: Dr S Vaidyanathan, Regional Spinal Injuries Centre, District General Hospital, Town Lane, Southport, Merseyside PR8 6PN, UK

been taken from patients with neuropathic when these patients underwent a therapeutic procedure in the urinary tract such as electrohydraulic lithotripsy of bladder stones or insertion of a ureteric stent. All patients had given written informed consent for taking bladder biopsies. The formalin-fixed, paraffin-embedded archival bladder biopsies ($n=46$) taken during 1994 and 1995 comprised the source for this pilot study. Histology of the available tissue revealed very little epithelium in three blocks, and therefore, they were excluded from further analysis. The remaining 43 biopsies of neuropathic bladder had been obtained from the following groups of patients: spinal cord injury 35, non-traumatic spinal cord paralysis 3, spina bifida 3, multiple sclerosis 2.

The control biopsies ($n=10$) were taken from the archival material. The control group represented patients with non-neuropathic bladder who had undergone routine bladder biopsy. The control group of bladder biopsies showed normal histology with no evidence of neoplasia of the transitional epithelium.

The important steps of immunohistochemistry are listed below:

(1) The sections were deparaffinised.
(2) Endogenous peroxidase activity was blocked by treating the sections with hydrogen peroxide in methanol for 12 min (12 ml of H_2O_2 in 400 ml of methanol).
(3) The sections were washed with deionised water.
(4) Trypsin digestion (2.7 g trypsin and 0.4 g of calcium chloride in 400 ml of Tris buffered saline (TBS) at 37°C for 20 min.
(5) The sections were racked into Sequenza Immunohistochemistry Staining Trays.
(6) The sections were treated with TBS for 5 min.
(7) Immunohistochemistry for sIgA was performed using rabbit polyclonal antibody against secretory component of IgA (DAKO catalogue no A0187). The primary antibody was diluted 1 : 50 in bovine serum albumin (BSA) and then applied over the sections for 10 min.
(8) The sections were washed twice for 4 min each, in TBS.
(9) Freshly prepared DAB was applied for 10 min.
(10) The sections were washed with deionised water for 4 min.
(11) The sections were transferred from Sequenza to metal staining racks, and then washed in running tap water for 2 min.
(12) Counterstain with haematoxylin.
(13) The tissue sections were dehydrated, and then mounted in resinous mountant.

Batches of controls were run while staining the biopsies from neuropathic bladder and the non-neuropathic bladder cases. Immunostaining for sIgA was performed contemporaneously on all tissue sections, ie those from neuropathic bladders and those from non-neuropathic bladders.

The presence of immunostaining in the vesical epithelium was recorded either as negative or positive. Those biopsies showing positive immunostaining were graded in a semi-quantitative manner: (i) faint; (ii) moderate; and (iii) strong. The pattern of immunostaining in the vesical epithelium was recorded: (i) cytoplasmic, or (ii) cell membrane. The location of the cells showing positive immunostaining was noted.

One of the assessors was blind to whether the section was a biopsy from a neuropathic bladder or from a non-neuropathic bladder.

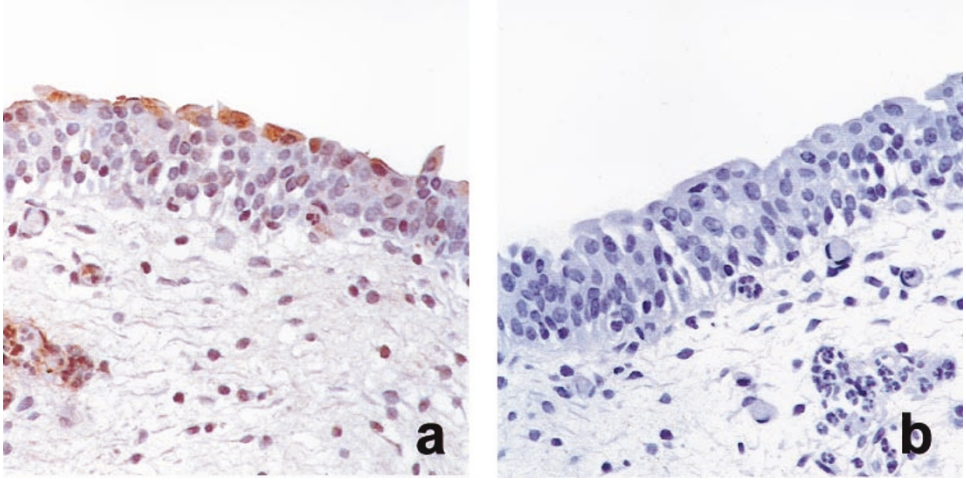

Figure 1 (**A**) Bladder biopsy from the control group: Immunohistochemistry for secretory Immunoglobulin A (sIgA) shows strong, cytoplasmic immunostaining in the superficial layer of cells of the transitional epithelium. (**B**) In contrast to **A**, a negative control shows no immunostaining for sIgA in the transitional epithelium

Results

All positive control sections showed strong, cytoplasmic immunostaining for sIgA in the superficial layer of cells of transitional epithelium (Figure 1).

The immunohistochemistry for sIgA in the vesical urothelium of biopsies, which were taken from patients with neuropathic bladder, showed variable staining pattern.

Squamous metaplasia was seen in five bladder biopsies. No immunostaining for sIgA was seen in all these five biopsies with squamous metaplasia (Figure 2A). (1) Strong immunostaining for sIgA was observed in the cytoplasm of the superficial cells of transitional epithelium in 14 patients (Figure 2B); (2) Moderate immunostaining was observed in the cytoplasm of luminal cells of vesical transitional epithelium in four biopsies; (3) Only faint immunostaining was present in the cytoplasm of superficial cells of the transitional epithelium in 16 biopsies (Figure 2C); (4) Three biopsies showed no immunostaining in the vesical transitional epithelium; (5) Intestinal metaplasia was observed in one biopsy; Immunohistochemistry for sIgA revealed cytoplasmic staining in the basal cells of the glandular structures.

To summarise:

(1) sIgA immunoreactivity was absent in squamous metaplasia.
(2) In 18 of 37 bladder biopsies in which transitional epithelium was present, moderate to intense immunostaining for sIgA was observed in the superficial cells of the transitional epithelium.
(3) In 19 of 37 biopsies, immunostaining for sIgA was either absent or faint in the transitional epithelium.

Discussion

It is possible that interruption of nerve supply to the urinary bladder may be associated with alterations in urothelial differentiation and maturation. The nervous system exerts a significant impact on lymphocyte distribution and function in certain mucosal tissues. Such a neural-immune interaction may affect the distribution, density, and output of IgA-containing cells in the mucosal tissues.

We carried out this pilot study with the archival materials of bladder biopsies taken from patients with neuropathic bladder and those with non-neuropathic bladder. We observed paucity of immunostaining for sIgA, in the transitional epithelium in 19 biopsies from patients with neuropathic bladder. This finding provides credence to the hypothesis that interruption of the nerve supply to the urinary bladder may lead to alterations in the urothelial functions.[2] One of the manifestations of such denervation-induced changes in the vesical urothelium could be the paucity of immunostaining for sIgA in the transitional epithelium as observed in 19 of 37 biopsies.

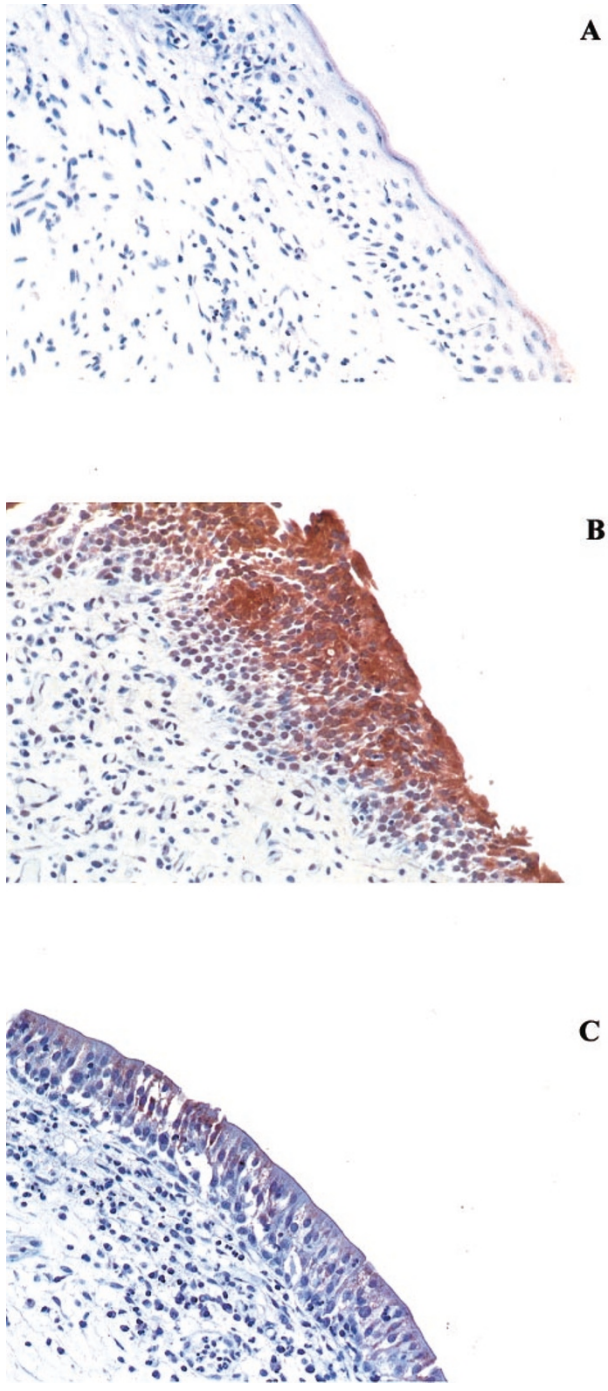

Figure 2 Immunohistochemistry for secretory Immunoglobulin A (sIgA) in vesical mucosal biopsies taken from patients with neuropathic urinary bladder. (**A**) Biopsy shows squamous metaplasia; immunostaining for sIgA is negative. (**B**) Strong cytoplasmic immunostaining is seen in the superficial cells of transitional epithelium. (**C**) Transitional epithelium shows only faint immunostaining for sIgA in this patient

This study raises certain issues that are relevant to the clinical management of spinal cord injury patients.

(1) What is the clinical significance of strong or weak immunostaining for sIgA in the urothelium of neuropathic bladder?
(2) Are spinal cord injury patients with absent or faint immunostaining for sIgA in the vesical urothelium at increased risk for developing cystitis?
(3) Does the intensity of immunostaining for sIgA in the urothelium of neuropathic bladder undergo progressive changes following spinal cord injury in a time-dependent manner?

A prospective, multi-centre study of spinal cord injury patients is warranted to investigate these important clinical issues. The participants should be enrolled soon after they have sustained spinal cord trauma and neuropathic bladder. This will facilitate accurate observation of the progressive changes in the mucosa of neuropathic bladder. Such a clinical trial should include recording of the occurrence of cystitis, 24 h intake of fluids, prescription of anti-bacterials, and method of drainage of the neuropathic bladder. Bladder biopsies can be obtained with flexible cystoscope every 12 months. Correlation of the clinical events with the findings of immunohistochemistry for sIgA in bladder biopsies taken annually, will yield answers to the queries raised above. The results of this prospective study will enable one to plan innovative therapeutic measures for prevention of bacterial cystitis in spinal cord injury patients.

References

1 Sirigu P, Perra MT, Turno F, Usai E. Immunohistochemical demonstration of secretory IgA in human urothelium. *Histol Histopathol* 1995; **10:** 645 – 650.
2 Vaidyanathan S *et al*. Possible role of denervation-induced changes in the urothelium in the pathophysiology of cystitis in patients with spinal cord injury: a hypothesis. *Spinal Cord* 1997; **35:** 708 – 709.