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The Effect of Pre-existing Bacteriuria on Bladder Resistance to Superinfection in the Rabbit

Philip M. Hanno, Robert Fritz*, Alan J. Wein and S. Grant Mulholland*

Division of Urology, Department of Surgery, University of Pennsylvania School of Medicine and the Philadelphia Veterans Administration Hospital, Philadelphia, Pennsylvania, USA

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Summary. Pre-existing bacteriuria of 2 to 3 weeks' duration in the rabbit had no effect on either the histological integrity of the sialomucin layer (anti-adherence factor) of the bladder mucosa or the protective effect of this layer against super-infection.

Key words: Urinary tract infection, Super-infection, Anti-adherence factor, Sialomucin layer.

INTRODUCTION

Previous studies performed in our laboratory (12-15) strongly suggest that the primary antibacterial defence mechanism of the normal bladder is the presence of a mucopolysaccharide substance on the luminal surface. This substance, shown to be a sialomucin by Monis and Dorfman (10, 11), is easily identified histologically by its PAS staining properties. It is probably produced within and secreted by the transitional epithelial cells. This sialomucin layer appears to prevent the adsorption of bacteria to the bladder mucosa, an important prerequisite for the establishment of a clinical cystitis (12-15). Bladders denuded of this layer are subject to massive attachment of inoculated bacteria (12-15). The normal anti-adherence protective function can be duplicated by the intravesical instillation of small amounts of exogenous mucopolysaccharide (3, 4).

On an a priori basis, it might be postulated that the presence of pre-existing, long-standing bacteriuria would alter the ability of this mechanism to protect the bladder against super-infection. To test this hypothesis, the study described below was undertaken.

*Current address: Department of Urology, Thomas Jefferson University School of Medicine, Philadelphia, Pennsylvania, USA

MATERIALS AND METHODS

Male New Zealand White rabbits weighing 2 to 3 kg were used. All animals were anaesthetised with Innovar (Pitman-Moore, Englewood, N. J.), 0. 2 mg per kg of body weight.

Induction of Urinary Tract Infection

The anaesthetised rabbit was secured and the perineal area was prepared with an antiseptic solution. Using a sterile technique, an 8 F catheter (Nelaton Catheter, ABCO Dealers, Inc., Milwaukee, Wis.) was inserted into the bladder per urethra. The bladder was allowed to drain.

Escherichia coli type 04 was transferred to Davis medium (Difco Laboratories, Detroit, Mich.), a minimal nutrient medium commercially available, by serial passage of 5% inocula (v/v) and incubation of the culture at 37°C overnight. One cubic centimeter of the culture was introduced into the bladder through the catheter and flushed in with 1 cc of 0. 9% NaCl. The catheter was removed. In order to estimate the number of colony-forming units (CFU) in the inoculum, a known fraction was serially diluted and cultured in brain-heart infusion agar (Difco Laboratories).

Every 3 days an attempt was made to obtain a urine specimen by the Credé technique and the resultant urine was cultured in brain-heart infusion agar, using serial saline and pour-plating methods, to estimate the number of bacteria per cubic centimeter. If the Credé technique was unsuccessful, the animal was given 100 cc of 0. 9% NaCl intravenously and urine was obtained by

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suprapubic needle aspiration with a 21-gauge needle using a sterile technique. Our primary concern was to determine whether a urinary tract infection existed ($>10^5$ CFU/cc) rather than to determine the absolute concentration of bacteria at the time. Two rabbits with consistently positive urine cultures for 3 weeks were sacrificed for histological study of the vesical mucosa, as were 2 normal rabbits, in order to examine the sialomucin layer.

Bacterial Adsorption Experiment

Eleven rabbits with bacteriuria of at least 2 weeks' duration were used in the following protocol. Eight uninfected rabbits served as controls.

Preparation of Bacteria. *Escherichia coli* type 04 was transferred to Davis medium by serial passage using 5% inocula (v/v) and incubation of the culture at 37°C overnight. To label the organisms with C-14, a 5% inoculum (v/v) was added to fresh Davis medium containing 10 μc/ml of ^{14}C-bicarbonate (Amersham-Searle, Des Plaines, Ill.), and the mixture was incubated at 37°C overnight. The culture was centrifuged at 3000 x g for 5 minutes and washed once with an equal volume of 0.9% NaCl. The bacteria were resuspended in 30% of the original volume of 0.9% NaCl and used immediately.

Colony-forming units were determined using brain-heart infusion agar as the growth medium.

Preparation of the Bladder. The rabbit was secured and given 100 ml of 0.9% NaCl intravenously over a 30-min period. A polyethylene tube (PE 205, Clay Adams, Parsippany, N.J.) was inserted suprapubically, secured with 3-0 silk pursestring suture, and brought out through the wound. Care was taken to manipulate the bladder as little as possible. The bladder was flushed through the cystotomy tube with four 15-ml aliquots of 0.9% NaCl.

Introduction of Bacteria. Under direct vision the bladder was emptied. Approximately 8 x 10^8 CFU of ^{14}C-labeled *E. coli* suspended in 0.5 ml of 0.9% NaCl were introduced into the bladder through the suprapubic tube and flushed in with 1 ml of 0.9% NaCl. With the catheter and the penis clamped, saline was given intravenously at a rate of 200 ml/h for 15 min. The suprapubic clamp was removed, and two 50-ml fractions of urine were collected during the ensuing diuresis. The animal was then sacrificed and the bladder removed. The mucosa was dissected free from the muscle layer and assayed for ^{14}C activity.

Recording of Radioactivity. Bladder tissue was homogenized overnight in 3 ml of 1.5 M NaOH at 60°C and neutralized with 2 N HCl. A known fraction of the homogenized tissue was suspended in 15 ml of Aquasol (New England Nuclear, Boston, Mass.), and radioactive counts were recorded by a Packard-Bell liquid scintillation counter. The radioactivities of bacterial samples suspended in 5 ml of Aquasol were determined in the same manner. Using the ratio of the bacteria viable cell count (CFU) to the C-14 uptake per ml of bacteria, we converted counts per min into the actual number of bacteria attached to the mucosa.

RESULTS

Light microscopic studies of the bladder mucosa of 2 pre-infected rabbits showed the PAS-staining layer to be intact and no different in appearance from those of the 2 control animals studied (Figs. 1, 2). On gross examination the infected bladders were oedematous, the mucosa being about twice as thick as control bladder mucosa, and more easily dissected from the underlying muscle layer.

The pre-infected and control groups had similar levels of bacterial adsorption following inoculation (Fig. 3). The mean number of radioactive bacteria adsorbed by the mucosa of the infected bladders was $5.18 \pm 8.60 \times 10^5$ compared with $4.81 \pm 6.60 \times 10^5$ organisms adsorbed by the normal bladders. The difference was not statistically significant ($P > 0.05$).

DISCUSSION

It has been postulated that the primary defence mechanism of the bladder resides in the integrity of the PAS-staining sialomucin layer on the luminal surface, which seems to prevent the adsorption of enough pathogens to cause a clinical infection of the bladder wall. The invading bacteria are thus confined to the urine by this protective mechanism, and subsequently flushed from the bladder by voiding (5, 9). Pre-existing chronic urinary infection (in this experiment, 2 to 3 weeks of continuous bacteriuria) might result either in exhaustion of the supply of sialomucin or in a compensatory increase in the production of this substance. The former condition might be expected to facilitate infection of the bladder wall by a subsequent inolculum, the latter to enhance the bladder's resistance to reinfection. Because of the extremely small numbers of bacteria adsorbed on the mucosa of the normal bladder after a massive inoculation, we would not expect our model to be sensitive enough to detect enough of a decrease in the number of adsorbed bacteria to show a statistically significant enhancement of any protective effect of prior infection. Previous studies (3, 4, 12-15) have shown a 10-fold increase in adsorption when the sialomucin layer was altered, and we would expect, therefore, to be able to document any major decrease in its protective function. It is clear that under the precise

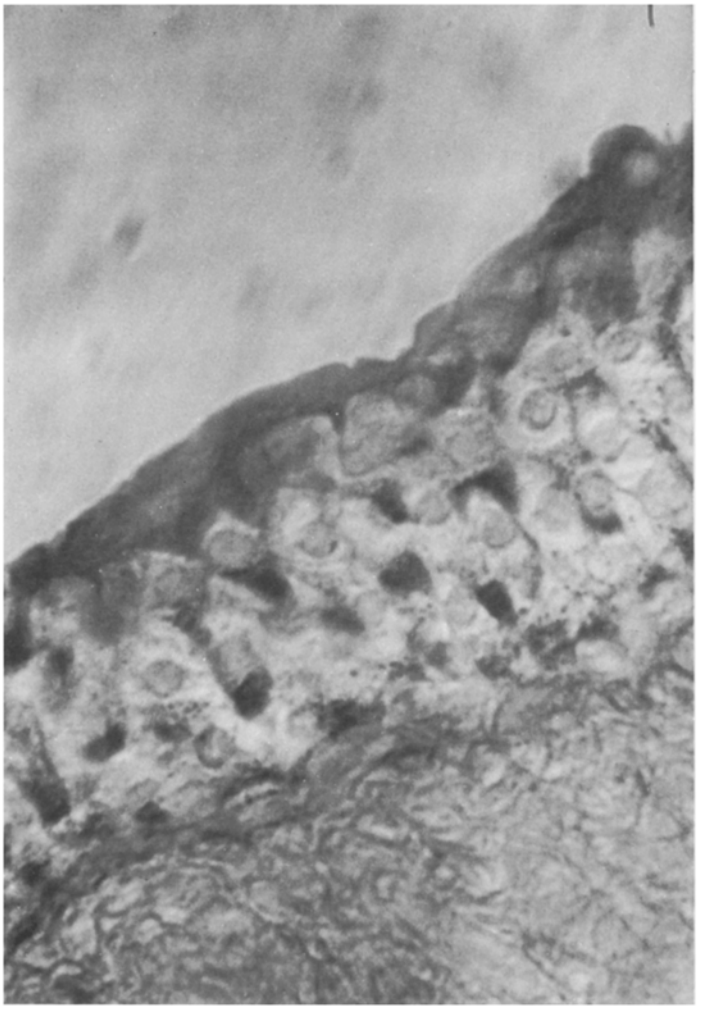

Fig. 1. Photomicrograph of a normal control bladder. Note the smooth PAS-positive luminal layer (PAS x 200)

Fig. 2. Photomicrograph of a bladder which had significant bacteriuria for 3 weeks. The integrity of the PAS-positive layer is intact (PAS x 200)

conditions of these experiments in the rabbit, bacteriuria of 2 to 3 weeks' duration had no deleterious effect on the histological integrity of the PAS-staining layer or on its ability to repel a subsequent massive bacterial challenge

In an interesting electron microscopic study of infected urothelium in two dogs and two humans, Lloyd-Davies et al. (8) showed that urinary infection increased the complexity of the bladder microcontour. They postulated that surface irregularity and increased adhesiveness might be important factors in reducing the ability of urinary washout to eliminate bacteria. Our findings appear to refute this hypothesis, at least with regard to the ability of the infected bladder to resist superinfection by a fresh, massive bacterial inoculation.

The incidence of bacteriuria has been reported to be elevated in women taking oral contraceptives and in postmenopausal women (2, 6, 17).

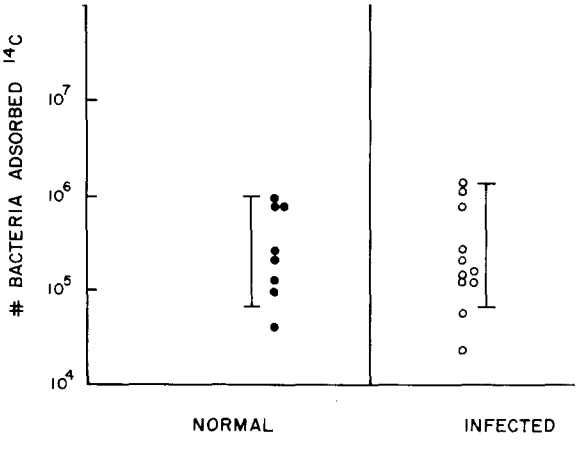

Fig. 3. Graphic representation of the number of radioactive bacteria adsorbed on control and infected bladder mucosas. One standard deviation of the mean is shown

Corriere et al. (1) demonstrated a significantly increased incidence of infection in women taking an oral progestogen, and believe that the increased incidence of bacteriuria in menopausal women may be an analogous phenomenon. It is conceivable that such hormones affect the bladder's antibacterial defence by altering the protective sialomucin layer of the bladder wall. Lapides (7) believes that ischaemia of the bladder wall due to overdistension and high intravesical pressures is the basic pathophysiological event causing a decrease in host resistance to infection. Again, it is possible that this hypothetical process could be mediated by a change in the sialomucin layer.

Uehling (16) has shown that pre-infection with Escherichia coli decreased subsequent adherence of Klebsiella pneumoniae in rat bladders. This occurred whether immunization was carried out with live or killed bacteria introduced intravesically and followed by a 6 week waiting period. All animals were uninfected at the time of the Klebsiella intravesical inoculation. It would seem this result is more likely due to a local immunity phenomenon than to any change in the sialomucin layer, as they reported no change in the bladder histology under light microscopy. Our study has shown no change in the sialomucin layer microscopically and certainly seems to rule out any increased susceptibility to bacterial adherence and superinfection in a bladder with an ongoing bacterial infection.

Parsons (14) has demonstrated that the anti-adherence activity of the vesical mucin is a generalised phenomenon by showing its ability to resist the adsorption of other strains of E. coli, Klebsiella pneumoniae, and Staphylococcus aureus. Thus, it is reasonable to believe that we may have obtained similar results in our experiment with another organism. In clinical practice, heavy bacteriuria, if it persists untreated, usually does so with the same organism over a period of weeks or even months. This correlates well with the data in our study which suggest that an infected bladder maintains its anti-adherence factor and thus its ability to defend against a second or third pathogen. Otherwise, we would expect most chronic infections to involve multiple organisms.

The 2 pre-infected rabbits sacrificed for histology showed an intact PAS-staining mucopolysaccharide layer in multiple sections. These rabbits were those with the longest duration of infection. The experimental data complement this finding, and it appears evident that the luminal mucoprotein is not generally affected under the precise conditions of this experiment.

Further studies are necessary to identify clinically significant factors or conditions capable of altering the sialomucin layer in such a way as to impair or enhance its protective function.

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Dr. P. Hanno
Department of Urology
5th Floor Silverstein Building
3400 Spruce Street
Philadelphia, Pennsylvania 19104
USA