The pathogenesis of urinary tract infections associated with *Escherichia coli*, *Staphylococcus saprophyticus* and *S. epidermidis*

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Summary. A model was developed in mice to study the pathogenesis of urinary tract infections caused by *Staphylococcus saprophyticus* strains LM-1 and LM-2, *Escherichia coli* SP444 and *S. epidermidis* E001. Murine urinary bladders were inoculated with $10^8$ bacteria via a temporary urinary catheter and samples of urine and bladder were removed at days 1, 3, 5 and 10 after inoculation. They were examined both bacteriologically and by electronmicroscopy. Severe disruption of the urothelium was present immediately and the urothelium had become oedematous and ulcerated with increased urothelial exfoliation. Disruption of the urothelium was similar with all the four pathogens studied. The three strains of coagulase-negative staphylococci preferentially attached to the urothelial cell tight junctions and were not associated with polymorphonuclear leucocytes. In comparison, *E. coli* SP444 was randomly attached over the entire urothelium and was often in association with macrophages. Phagocytosis of *E. coli* by superficial urothelial cells also occurred.

Introduction

Establishment of a urinary tract infection (UTI) depends upon the interaction of bacterial virulence factors with the host. Similarly, maintenance of a UTI is influenced by the interaction between the pathogen and the urothelium. In many in-vitro studies of bacterial attachment to urothelial cells, it has been assumed that the urothelium remains intact and offers a consistently uniform surface for attachment. However, in-vivo studies of acute, chronic and catheter-associated UTI in patients have demonstrated that, during a UTI, the urothelium is disrupted and disorganised with exfoliation of superficial urothelial cells exposing the underlying immature cells. Preferential colonisation of these immature cells has also been described.

In the present study a murine model of unobstructed ascending UTI was used to compare the interaction between the urothelium and three species pathogenic for the urinary tract—*Escherichia coli*, *Staphylococcus saprophyticus* and *S. epidermidis*. The colonisation and response of the urothelium to challenge with these bacteria was examined and compared by the use of bacteriological and ultrastructural techniques.

Materials and methods

Bacterial strains

Two strains of *S. saprophyticus* (LM-1, LM-2) and one strain each of *S. epidermidis* (E001) and *E. coli* (SP444) were used. The staphylococci were identified by gram staining, catalase, coagulase and DNAase reactions. The *S. saprophyticus* strains were further identified by resistance to novobiocin (5 mg/L; Sigma) and by API STAPH (API Systems) and fluorogenic substrate tests. The *E. coli* strain was characterised by standard biochemical tests.

Slime detection

Christensens test for the detection of slime and the serum soft agar method were used to determine the presence of a capsular or slime layer on the surface of the coagulase-negative staphylococci (CNS) grown to stationary phase at 37°C in Tryptone Soya Broth (Oxoid).

Detection of P and Type 1 fimbriae

*E. coli* SP444 was tested for the presence of both P and Type 1 fimbriae. Bacteria were grown on 5% Horse Blood Agar (Gibco) and emulsified in phosphate buffered saline.

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(PBS; pH 7-2) before testing. The presence of Type 1 fimbriae as indicated by mannose sensitive agglutination of guinea-pig erythrocytes, was ascertained by the method of Parry et al.10 The presence of P fimbriae was determined by a latex particle agglutination test (Bach Test, Kabivitrum).

Mouse urinary tract infection model

Female Parkes mice, aged 3 months and weighing approximately 30 g, were supplied by the Animal Unit, National Institute for Medical Research, London. The animals were allowed several days to return to a normal drinking and diuresis pattern after their journey to the laboratory from London.

Bacteria were grown to stationary phase in Tryptone Soya Broth at 37°C for 18 h before inoculation. Mice were anaesthetised with halothane (May and Baker Ltd) 3% in oxygen (British Oxygen Company) and the perineum of each animal was sprayed with chlorhexidine (Stuart Pharmaceuticals Ltd) 2-5%. A sterile blunt 23 gauge needle (Sherwood Medical) was used as a catheter and was introduced 1-3 mm into the urethral meatus. The catheter was passed up the urethra, around the pubic symphysis and advanced into the urinary bladder. Any residual urine present in the bladder was gently withdrawn into a 1-ml syringe attached to the catheter. The syringe was then replaced with another containing the bacterial suspension (109 bacteria/ml) and 0-1 ml was introduced into the urinary bladder via the catheter. Immediately after inoculation the catheters were removed and the animals were allowed to recover. The mice were provided with glucose (Phoenix Pharmaceuticals Ltd) 5% in water to maintain an osmotic diuresis.11 The animals were killed at various time intervals by cervical dislocation and samples of urine and bladder were obtained immediately. Some urinary bladders were studied by scanning electronmicroscopy, others were examined bacteriologically after homogenisation in Brain Heart Infusion Broth (Oxoid).

Bacteriology

Tissue homogenates and urine samples were inoculated on to MacConkey Agar (Lab M) for the isolation of E. coli SP444, Mannitol Salt Agar (Difco) to isolate the CNS strains and DST Agar (Oxoid) containing novobiocin (5 mg/L) to isolate selectively S. saprophyticus LM-1 and LM-2.

Scanning electronmicroscopy (SEM)

Tissue samples were fixed immediately in glutaraldehyde 3% in 0-1 M cacodylate buffer (BDH Ltd) for up to 18 h and then dehydrated in increasing concentrations of alcohol. Finally the samples were placed in acetone as previously described.12 The tissue was then dried in a critical point dryer (Polaron Equipment) and coated with gold for 2 min in a sputter coater (EM Scope) as previously described.13 The specimens were examined in a Cambridge S600 stereoscan electronmicroscope.

Thin sectioning

Urinary bladders were fixed in glutaraldehyde 3% in 0-1 M cacodylate buffer at 4°C for up to 18 h and processed as previously described.12 Thin sections (50–60 nm) were cut with an ultramicrotome (Ultracut E, Reichart Jung), mounted on formvar coated grids and stained with osmium tetroxide and uranyl acetate.13 The sections were viewed with a Joel 100S transmission electronmicroscope.

Results

Adherence factors

The presence of a capsular or slime layer was detected on the surface of S. saprophyticus LM-2 and S. epidermidis E001 by both the Christensen tube test and the serum soft agar method. No capsule or slime layer was associated with S. saprophyticus LM-1. E. coli SP444 had both P fimbriae (positive latex test) and Type 1 fimbriae as indicated by mannose sensitive agglutination of guinea-pig erythrocytes.

Ultrastructure results

The ultrastructure and bacteriology of the normal murine urinary tract was investigated in a group of eight female Parkes mice. There was no growth of bacteria from urine samples or the bladder tissue from any of these control animals. Four of the eight bladders taken from the control animals were examined by SEM. The normal SEM appearance of the murine non-distended urinary bladder with folded urothelium is shown in fig. 1. Convoluted microridges (1-2 μm diameter) covered the bladder folds and obscured the boundaries between the individual urothelial cells lining the urinary lumen. Only occasional organised urothelial exfoliation involving single cells was detected in all the normal bladders.

Following the inoculation of each of the four species of bacteria under study, the urothelial response was similar. After one day the urothelium became oedematous with generalised swelling associated with the loss of microplications. During the following 4 days, an increasing number of mature urothelial cells were exfoliated revealing the immature urothelium below (fig. 2). The exfoliation was disorganised with relatively large numbers of cells involved in many areas of the urothelium. The topography of the exfoliated cells also appeared more irregular. The cellular damage
Fig. 1. Scanning electronmicrograph of the normal murine urothelium showing the convoluted microridges and obscured tight junctions of the epithelium. Bar = 10 μm.

Fig. 2. Scanning electronmicrograph showing the damaged urothelium and exfoliation of mature epithelial cells. Bar = 4 μm.
was generalised and occurred in the presence of all the bacteria tested.

Bacteria adhered to the bladder wall within 24 h of inoculation with all four bacterial species studied. *E. coli* SP444 cells were attached in groups of up to several hundred which were randomly distributed across the urothelial surface. Colonisation was not confined to any particular type of urothelial cell nor to a specific area of individual cells (fig. 3). In comparison, all the CNS studied initially formed randomly distributed but discrete microcolonies on the urothelial surface. However, 3 days after inoculation, *S. saprophyticus* strains LM-1, LM-2 and *S. epidermidis* E001 displayed a predilection for the urothelial tight junctions (fig. 4) which became increasingly apparent during the next 7 days. There was no evidence of selective adherence of *E. coli* SP444 in any of the experiments.

A phagocytic response was observed within 24 h of inoculation of *E. coli* SP444 with bacteria at various stages of engulfment. A few macrophages (fig. 5) and also superficial urothelial cells (fig. 6) contained intracellular organisms. The macrophages were mainly associated with the larger colonies of *E. coli* SP444. This phagocytic response was not evident when the murine bladder was challenged with any of the CNS strains studied, independent of the expression of a capsular polysaccharide layer.

**Bacteriology**

Isolation of the test organisms from either urine or bladder samples was considered to be suggestive of an established infection (table). At day 1 after inoculation, both strains of *S. saprophyticus* and *E. coli* SP444 were present in bladder samples. However, *S. epidermidis* E001 was not present in sufficient numbers to be detected in the bladder until 3 days after inoculation.

**Discussion**

The results of this study have illustrated that an experimental UTI can be induced by direct inoculation of the murine bladder without the use of additional obstructive or surgical techniques. Furthermore, the sequence of events in the bladder in response to challenge with *S. saprophyticus* LM-1 and LM-2, *S. epidermidis* E001 and *E. coli* SP444 have been compared for the first time.

When each of the four bacterial strains studied

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**Fig. 3.** Scanning electronmicrograph showing the murine urothelium colonised by *E. coli* SP444 3 days after challenge. Surface structures, possibly aggregated pili, are present on the bacteria and appear to be involved in attachment. Bar = 4 μm.
was introduced into the urinary bladder, generalised urothelial damage occurred within 3 days of challenge. These findings correspond to those of previous studies of a rat model of UTI, in which a urothelial response consisting of oedema, swelling, disruption of intercellular junctions and exfoliation of urothelial cells occurred 2 days after challenge with *E. coli*. Similarly, studies of the human urothelial response to UTI have reported ulceration and increased exfoliation of mature urothelial cells, particularly in chronic UTI. Conversely, Sobel and Vardi failed to detect any structural changes to the rat urothelium despite colonisation with *Pseudomonas aeruginosa*.

Therefore, it would appear that the bladder urothelium can be damaged in response to infection but the degree of change may be related to factors such as length of infection as well as the invading bacterial species. This suggestion is illustrated by the present observations that a pathogenic strain of *E. coli* as well as a strain of *S. saprophyticus* were both able to establish immediately an acute UTI in the murine model. On the other hand *S. epidermidis*, similarly inoculated, required a further 3 days before being detected by conventional bacteriological tests. This result further suggests that *S. saprophyticus* has a similar pathogenic potential to *E. coli*, offering an explanation why both of these organisms are relatively common causes of UTI.

Bacterial multiplication within the urinary tract is limited by the “washout” effect of urine and the antibacterial activity of the bladder mucosa. Cobbs and Kaye suggested that this antibacterial effect was due to macrophages associated with the urothelium. This was confirmed in the present study in which polymorphonuclear leucocytes, actively phagocytosing bacteria, were seen closely adherent to the urothelium. The urothelial cells also appeared to be engulfing bacteria by phagocytosis. This observation corresponds to those of Fukushi.
et al.\textsuperscript{15} and Racz et al.\textsuperscript{19} who have also previously reported the presence of bacteria within phagosomes of urothelial cells. The exfoliation of urothelial cells, which have a phagocytic role, may indeed be a defence mechanism, eliminating bacteria from the urinary tract.

The extent and distribution of bacterial colonisation of the mouse urothelium was shown in the present study to be dependant upon bacterial species. \textit{S. saprophyticus} strains LM-1, LM-2 and \textit{S. epidermidis} E001 displayed an affinity for the tight junctions of the urothelium. This characteristic was not associated with \textit{E. coli} and may reflect a previously unrecognised adherence mechanism which is peculiar to the pathogenesis of CNS UTI. Balish et al.\textsuperscript{20} described a layer of mucin covering the urothelia of rats which reduced the adherence of \textit{E. coli} to the bladder wall in a non-specific manner. A similar material, partially covering the human urothelium, has also been reported.\textsuperscript{21} This substance may account for the irregular distribution of adherent \textit{E. coli} observed in this study, although no structure resembling a mucin layer was seen by SEM.

Despite this apparent selectivity of attachment, when challenged with any of the three bacterial species studied, resultant damage of the urothelium was universally extensive and generalised. The mechanisms of this change are currently unclear although it seems likely that production of extracellular products may play a role in the pathogenesis of these infections. For example, \textit{S. saprophyticus} synthesises a urease which is transported extracellularly. Urease production by bacteria such as \textit{Campylobacter pylori} has been linked to cytotoxic changes\textsuperscript{22} and this may be occurring in \textit{S. saprophyticus} UTI. Conversely, some of the urothelial damage may have simply been the result of the inflammatory response and associated oedema.

Whatever the mechanism is for the urothelial damage observed in this murine model, it appears to correspond closely to that previously observed in man.\textsuperscript{1} Therefore, the mouse unobstructed UTI model offers a relatively simple method to test \textit{in vivo} some of the potential virulence factors and to elucidate further the interaction between urinary tract pathogens and the urothelium.

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**Table.** Bacteriological analysis of urine samples and bladder tissue homogenates illustrating the incidence of UTI induced as a result of direct challenge with \textit{E. coli}, \textit{S. saprophyticus} and \textit{S. epidermidis}

<table>
<thead>
<tr>
<th>Bacteria tested</th>
<th>Site</th>
<th>Percentage of animals infected on days* after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>\textit{S. saprophyticus} (LM-1)</td>
<td>Bladder</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>NA</td>
</tr>
<tr>
<td>\textit{S. saprophyticus} (LM-2)</td>
<td>Bladder</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>100</td>
</tr>
<tr>
<td>\textit{S. epidermidis} (E001)</td>
<td>Bladder</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>NA</td>
</tr>
<tr>
<td>\textit{E. coli} (SP 444)</td>
<td>Bladder</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>83</td>
</tr>
</tbody>
</table>

*NA, not available for bacteriological tests.

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**REFERENCES**

11. Keane W F, Freedman L R. Experimental pyelonephritis XIV. Pyelonephritis in normal mice prolonged by...


