

699

Horsley H¹, Kupelian A¹, Holland D², Gill K¹, Brackenridge L¹, Sathiananthamoorthy S³, Malone-Lee J¹

1. University College London, UK, 2. University College London Medical School, UK, 3. Royal Veterinary College, UK

COLOUR SNAPSHOTS OF DISEASE IN ACTION IN PATIENTS WITH OAB

Hypothesis/aims of study

There is now a considerable body of evidence indicating that in some individuals, chronic urinary tract infection (UTI) underlies overactive bladder (OAB) symptoms. Murine studies have shown intracellular colonisation of uroepithelial cells by *E.coli* in the pathogenesis of UTI.

Three distinct phases of this pathological process are recognised: (i) **Early**: Loose collections of adherent rod like bacteria collect on and below the cell membrane. (ii) **Middle**: Bacteria divide, developing coccoid morphology, forming biofilm-like pods protruding from the cell (iii) **Late**: Bacteria develop flagellated, rod-shaped morphology prior to pod rupture (1,2).

Microbiological studies have identified intracellular colonisation in some patients with OAB symptoms (3) which at present remains uncorroborated by imaging. The innate immune response causes urothelial shedding providing a rich urinary source of potentially colonised cells.

This study developed a confocal microscopic technique for scrutinising urinary uroepithelial cells. A comparative observational study tested its clinical relevance.

The challenges associated with this study included: 1: Achieving differential staining of the epithelial cell membrane and bacteria; 2: Ensuring that the bacterial stain entered the cell whilst keeping the membrane intact; 3: Achieving full cell membrane staining of all surfaces; 4: Avoiding excessive flattening of the cells by centrifugal forces associated with centrifugation during cell preparation; 5: Working with dead epithelial cells, as early fixation was needed to prevent excess bacterial growth after sampling.

Study design, materials and methods

Patients with OAB symptoms were assessed by blinded clinicians. Meticulous midstream urine samples (MSU) were analysed and a microscopic epithelial cell and white blood cell count (cells μl^{-1}) enumerated with a haemocytometer. The final labelling protocol was devised following an exhaustive series of pilot experiments.

Labelling protocol: 1: Prompt fixation of 1ml urine with 4% formaldehyde at 4°C for 30 minutes 2: Cell membranes labelled in solution with 5ul wheat germ agglutinin conjugate Alexa-Fluor 488 at room temperature for 10 minutes 3: Cytocentrifuge to a slide at 200rpm for five minutes 4: Circumscribe resultant cellular deposit using hydrophobic barrier pen 5: Wash deposit with Hank's Balanced Salt Solution for five minutes and repeat three times 6: Mount deposit with VectaShield with DAPI 7: Affix coverslip.

Microscopy: 1: Upright epifluorescent microscopy to enumerate the proportion of infected epithelial cells within the cellular deposit 2: Production of cross-sectional Z-stacks via confocal microscopy to analyse intracellular pathological phenomena.

Results

Confocal cross-sectional micrographs, obtained from infected cells, exhibited all of the key stages of the pathological process described in the introduction (**See Figs. 1-3**).

30 female patients with OAB (mean age=52; sd=17) and 10 asymptomatic female control subjects (mean age=38; sd=15) were included in the analysis. A significant difference in the proportion of infected uroepithelial cells was observed between the groups (Kruskal-Wallis test; $H=4.31$, $df=1$, $p=0.038$). This study had a greater than 80% power to detect a significant between group difference ($\alpha=0.05$).

Interpretation of results

Confocal images from OAB patients demonstrated remarkable similarities to pathology previously reported in the studies of acute UTI in mice and humans, including: **A**: Adherent rod-like bacteria (**Fig.1**); **B**: Uptake of bacteria by the formation of endosomes, coccoid morphology (**Fig.2**); **C**: A tightly packed, biofilm-like pod of intracellular bacteria (**Fig.3**).

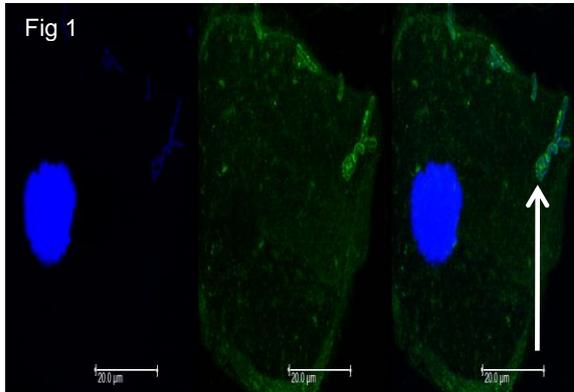


Fig.2 Dual channel, cross-sectional, confocal image of an infected, shed uroepithelial cell. The image demonstrates uptake of bacteria by formation of endosomes and coccoid bacterial morphology. Cropped, 630X magnification, stained with VectaShield/DAPI and Alexa-Fluor.

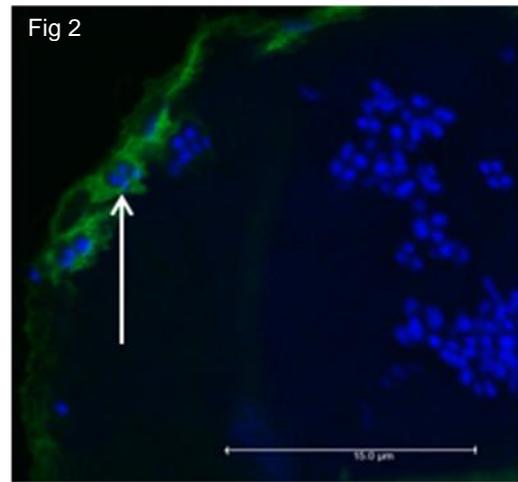


Fig.3 Dual channel, cross-sectional, confocal image of an infected, shed uroepithelial cell. The image demonstrates a tightly packed, biofilm-like, pod of intracellular bacteria. 630X magnification, stained with VectaShield DAPI and Alexa-Fluor).

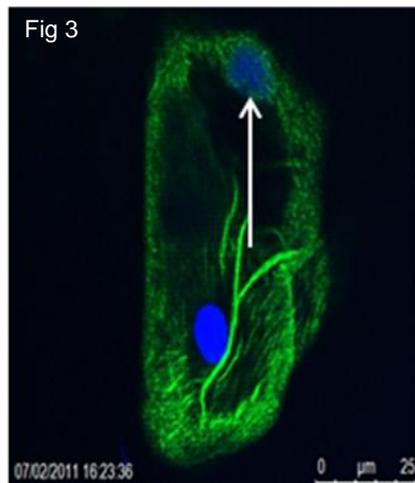


Fig.1 A dual channel, confocal image of an infected, shed uroepithelial cell showing adherent rod-like bacteria. 630X magnification. The three images are differentially stained (left image, VectaShield DAPI; centre image, Alexa-Fluor; right image, VectaShield DAPI and Alexa-Fluor).

Concluding message

Confocal microscopy demonstrated that urothelial cells from patients with OAB manifest all the stages of bacterial invasion, which to date have been described only in acute urinary infection. This is excellent evidence of a hitherto unrecognised pathology in OAB.

References

1. Proc Natl Acad Sci U.S.A. 2006; 103(52): 19884-89.
2. PLoS Med 2007; 4: 329.
3. PhD Thesis 2010 (Held on file)

<i>Specify source of funding or grant</i>	None
<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	HUMAN
<i>Was this study approved by an ethics committee?</i>	Yes
<i>Specify Name of Ethics Committee</i>	Whittington and Moorfields Research Ethics Committee
<i>Was the Declaration of Helsinki followed?</i>	Yes
<i>Was informed consent obtained from the patients?</i>	Yes