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CAUGHT INFLAGRANTE – BACTERIA FROM OAB PATIENTS INVADE UROTHELIAL CELL LINES

Hypothesis / aims of study

To imply a role for bacterial infection as an important part in the aetiology of OAB, and propose that such infections miss detection by routine urine testing methods, is a bold claim. If correct, it will greatly perturb our understanding of this unpleasant condition. It is entirely reasonable that such hypotheses should be submitted to rigorous repetitive testing and that any implied mechanisms be explored exhaustively.

Recent data have clearly implicated intracellular bacterial colonisation of the urothelial cells in the pathological mechanisms of acute urinary tract infection causing frequency dysuria. It has been discovered that in a murine model of UTI, populations of *E. coli* can persist in the bladder for months on end, during which time they exist as a quiescent reservoir of infection. Because the bacteria are located inside the cells they can increase in number while evading the action of polymorphonuclear leucocytes (PMNs) as well as antibiotics and other defence mechanisms. There are intracellular innate immune mechanisms, notably the fusion of the bacteria containing endosomes with lysososmes but it seems that some microbes have evolved methods of escaping such attacks. Intracellular colonisation would be a most apt mechanism for achieving a chronic infection that was hard to detect, but nevertheless sufficient to cause OAB symptoms.

Culture of the urinary cellular sediment in OAB patients has improved the ability to isolate bacteria from patients with OAB symptoms and negative routine urine cultures [(1)]. Comparisons with normal asymptomatic controls indicated that these isolates were part of a pathological process. Given intracellular colonisation, it would not be surprising that culturing the concentrated cell sediment proved better at isolating pathogens. A key criterion for validity would demand that the putatively pathological isolates could be shown to invade sterile urothelial cell lines.

This study was designed to test the hypothesis that microbes isolated from the urine of patients with OAB symptoms could be shown to be capable of invading and colonising a sterile cultured uroepithelial cell line. By contrast, microbes obtained from asymptomatic controls would not be capable of invasion. The premise was that intracellular colonisation of uroepithelial cells by pathogens could be incriminated in the pathogenesis of OAB.

Study design, materials and methods

With ethical committee approval, we studied four bacterial isolates, previously obtained and stored frozen, from patients presenting with symptoms of OAB. Additionally, a *Lactobacillus.gaseri* isolate, obtained from an asymptomatic control volunteer, was used. A bladder epithelial cell line from a transitional cell carcinoma (EJ138) was grown to confluency in Eagles Minimal Essential Medium. Once confluent, the cells where infected with either *Eschericia. coli, Enterococcus. faecalis*, *Streptococcus. angiosus, Proteus. mirabilis or Lactobacillus.gaseri* with a multiplicity of infection of 100 bacteria to 1 epithelial cell. After 2 hours of incubation, the cultures were incubated with gentamicin 200µg/ml to kill any extracellular bacteria. All the bacteria had previously been shown to be fully gentamicin sensitive. After a further 24 hours, the epithelial cells were washed and lysed with Triton X 0.1%. Intracellular bacteria were enumerated by culture of the medium five minutes after lysis.

The difference between groups were analysed by means of the non-parametric Mann-Whitney U test for independent samples.

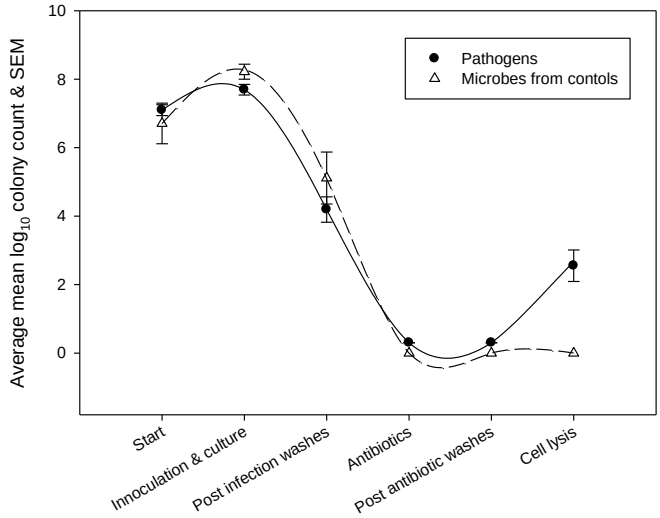
Results

The data comparing all the pathogenic bacteria from patients with the commensal *Lactobacillus.gaseri* from the control are illustrated in the figure. The key is the dissociation of the curves in respect of the colony count immediately after lysis. The rise in the patient bacteria curve incriminates intracellular colonisation, so the bacteria inoculated into the cell culture rapidly colonised the cells. The *Lactobacillus.gaseri* obtained from the normal controls did not do this. The average bacterial counts post cell lysis (intracellular bacterial count) were: *E.coli* 1.8x10 cfu ml^{-1}, *E.faecalis* $1.02x10^4$cfu ml^{-1}, *Strep.anginosus* $2.69x10^3$cfu ml^{-1}, *Proteus.mirabilus* 3.20 $x10^3$cfu ml^{-1}, *Lactobacillus. Gaserri* 0 cfu ml^{-1}. These differences were statistically significant (p=.036 95%CI difference = 10^{10} to 10^1 cfu ml^{-1}) but the clinical significance is well illustrated by the mean difference of 10^6 cfu ml^{-1}

Interpretation of results

This study challenged apparent pathogens isolated from patients with OAB symptoms to invade sterile cultured urothelial cells. The bacteria were *E.coli, E.faecalis Strep.anginosus,*and *Proteus.mirabilus,* Until now the accepted wisdom was that *E.coli* was the only pathogen with these properties. This is the first time that these phenomena has been shown to contribute to the pathology of OAB symptoms. This is indeed a new departure.

Cell line culture innoculation and invasion assays
Pathogens and microbes isolated from controls



Stages in the innoculation and invasion assay

Concluding message

It is possible, that cell invasion and a reservoir of intracellular bacteria play a role in the persistence of OAB. It is important that other species additional to *Eschericia. Coli* have, for the first time, been implicated in this pathology.

References

1. Neurourol.Urodyn., 2009; 8, 779 - 780.

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