THE ANTIMICROBIAL PEPTIDE AND MAST CELL ACTIVATOR, BETA DEFENSIN 2 (BD2) IS PERSISTENTLY EXPRESSED IN THE BLADDER OF WOMEN WITH INTERSTITIAL CYSTITIS

Hypothesis / aims of study
There is increasing evidence that individuals with Painful Bladder Syndrome / Interstitial cystitis (IC) have a defect in the protective function of the bladder Glycosaminoglycan (GAG) mucous layer which protects the bladder surface from both urinary toxins and infection. The aetiology of this defect is not known but one proposed mechanism is that an inherited or acquired defect in the innate host epithelial defence mechanisms results in an abnormal response to infection causing injury to the epithelial GAG layer and leading to an on-going permeability defect with lasting damage to the barrier function of urothelium. Within the human urinary tract, the innate immune response is characterised by the constitutive or inducible expression of antimicrobial peptides (AMPs). These small cationic peptides are critical to host anti-microbial defence. In Crohn’s disease, which like IC is also thought to involve a defect in the GAG layer, altered expression of AMPs has been demonstrated in the gut. We therefore proposed that a similar defect in the expression of AMPs within the bladder may be implicated in interstitial cystitis. We hypothesised that women with IC have altered expression of endogenous AMPs resulting in an aberrant immune response which leads to a breakdown in the epithelial GAG layer.

Study design, materials and methods
Following ethical approval and institutional review, we recruited 10 female patients with IC defined by the European Society for the Study of Interstitial Cystitis (ESSIC) criterion and compared bladder biopsies and overnight urine collections with samples collected from a similar population of 8 women with active UTI and 31 control patients with no disease. Biopsies were analysed using qRT-PCR to measure expression of four urogenital AMPs: Beta Defensin 1 (BD1), Beta Defensin 2 (BD2), Human alpha-Defensin 5 (HD5) and Cathelicidin (LL37). Peptide translation was confirmed using immunohistochemistry of biopsies and sandwich ELISA of the urine samples. Urine samples were cultured to check for infection status. Statistical analysis was carried out using t-test with Welch’s correction for unequal variance.

Results
Comparison of expression of BD1, HD5 and LL37 expression showed no difference between controls and IC patients. However, biopsies taken from IC patients showed significantly higher expression of BD2 (3.78 ± SEM 0.95 AU) compared with controls (0.25 ± SEM 0.09 AU), p<0.01. Biopsies obtained from areas with Hunner’s ulcers exhibited the highest levels of BD2 expression (215.1 ± SEM 77.57 AU).

On analysis of urine samples, low levels of BD-2 were detectable in the urine of healthy controls subjects (10.8 ± SEM 0.79 pg/mL) but these levels were significantly increased in IC patients (34.38 ± SEM 3.72 pg/mL) and were even greater in patients with acute urinary tract infection (88.92 ± SEM 20.31 pg/mL), p<0.01.

Interpretation of results
These data suggest that secretion of BD2, a potent anti-microbial, is significantly increased in IC patients and that this response has similarities to that seen during urinary tract infection. This indicates that despite the absence of infection in IC, the bladder is still mounting a response akin to that seen during cystitis with appreciable amounts of BD2 released in the bladder and expression of BD2 appears to correlate with the magnitude of the inflammation. As well as acting as an antimicrobial, BD2 is recognised as a mast cell activator and chemotractant which may provide clues to the origin of the mastocytosis commonly seen in IC urothelium and the damage caused by their activation.

Concluding message
The antimicrobial peptide, BD2 appears to play a key role in an aberrant chronic innate immune response similar to that seen acutely during infection – this may be an important underlying mechanism for the urothelial pathology seen in IC.

Disclosures
Funding: Cystitis and Overactive Bladder Foundation Wellcome Trust Clinical Trial: No Subjects: HUMAN Ethics Committee: NHS National Research Ethics Service (County Durham and Tees Valley Research Ethics Committee. REC Reference 09/H0905/15 Helsinki: Yes Informed Consent: Yes