MAST CELLS IN THE URINARY BLADDERS OF INTERSTITIAL CYSTITIS/BLADDER PAIN SYNDROME PATIENTS EXPRESS THE MAST CELL RELATED G-PROTEIN COUPLED RECEPTOR X2 (MRGPRX2): POTENTIAL ROLE IN THE PATHOGENESIS OF NEUROGENIC INFLAMMATION.

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HYPOTHESIS / AIMS OF STUDY

Interstitial cystitis/Bladder pain syndrome (IC/ BPS) is a chronic debilitating inflammatory disease of the urinary bladder that significantly affects the quality of life. Recent reports have shown progressive increase in the prevalence and incidence rates of IC/BPS across genders. This places IC/BPS amongst conditions that merit intensive research to bridge the wide gaps between diagnosis and treatment. The pathophysiology of IC/BPS is elusive and rather multifactorial, in which a defect in the bladder urothelium and chronic inflammation of bladder wall are key features. The numbers and activity of mast cells, in addition to the density of the substance P (SP) positive nociceptive sensory nerve endings, are significantly elevated in the bladders of IC/BPS patients. The urothelial barrier defect is associated with increased urothelial permeability, allowing harmful urinary constituents to penetrate the bladder wall and depolarise the nociceptive sensory nerve fibres, which release neuroactive substances including SP, inducing mast cell degranulation and tissue inflammation. However, the responsiveness of mast cells to SP is variable between different tissues depending on the local cellular micro-environment. MRGPRX2 is a recently identified G-protein coupled receptor involved in mast cell responsiveness to SP in different chronic inflammatory conditions, and its expression is associated with increased tissue inflammation. The responsiveness of the bladder mast cells to SP remains poorly understood. This makes theories related to neurogenic inflammation in the pathogenesis of IC/BPS uncertain. Thus, the potential contribution of neurogenic inflammation in the pathogenesis of BPS/IC warrant further investigation.

STUDY DESIGN, MATERIALS AND METHODS

In the present study, we investigated the responsiveness of bladder mast cells to SP through studying the expression of MRGPRX2 on BPS/IC bladder mast cells. Formalin Fixed Paraffin embedded urinary bladder biopsies from 18 IC/BPS patients, in addition to healthy controls from 10 bladder biopsies with non-invasive bladder cancer. These were obtained from the human tissue archive, clinical pathology department, University Hospital Southampton, NHS FT. IC/BPS were diagnosed clinically based on the ESSIC criteria. 4 serial sections, each of 4μm thickness, were cut from each bladder biopsy and stained with antibodies against mast cell-specific proteases tryptase (AA1) and chymase (CC1), as well as anti-MRGPRX2 (Abcam, USA). All staining was performed using an automated autostainer platform. Following microscopic scanning, images from the serial sections were aligned and superimposed using Adobe Photoshop CS6 software. Mast cell quantification and co-expression patterns were studied using Image J software. Statistical differences between the IC/BPS and the control groups were analysed by Mann Whitney U test and unpaired t-tests using GraphPad Prism 9 software.

RESULTS

All of the 18 IC/BPS biopsies consistently co-expressed MRGPRX2 with tryptase and chymase in the lamina propria and detrusor layers of the bladder wall, indicating the potential for bladder mast cells to respond to SP (Figure 1). Tissue resident mast cells are found in 2 subtypes of different protease expression behaviour, mast cells tryptase only (MCT) and mast cell tryptase chymase (MCTC). The average density of the two mast cell subtypes MCT and MCTC in the IC/BPS group was 177 and 167 cells/mm2, respectively. This was significantly higher when compared to the control group. Moreover, the percentage of mast cells that co-expressed tryptase and chymase (MCTC), out of all mast cells (tryptase +ve) in the IC/BPS group averaged at 67%, which was significantly higher compared to the controls ($P < 0.05$). Interestingly, about 66% of the tryptase +ve mast cells (all mast cell) expressed MRGPRX2, which was significantly higher compared to the control group (25%; $P < 0.0001$). Similarly, about 65% of the mast cell stained with tryptase and chymase (MCTC) expressed MRGPRX2, which was significantly higher compared to the controls (30%; $P < 0.0001$) (Figure 2).

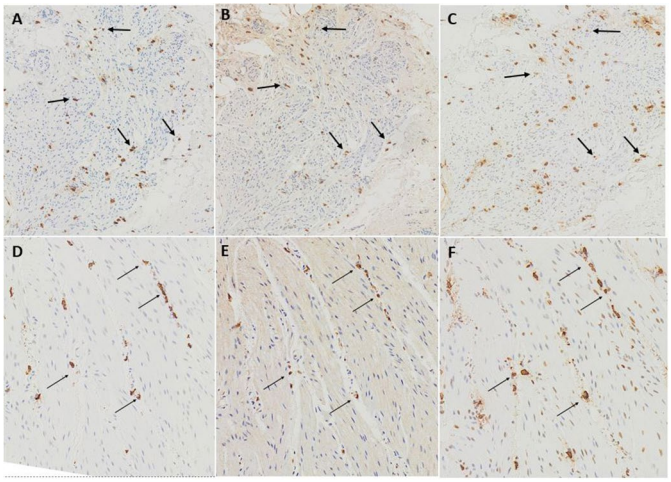
INTERPRETATION OF RESULTS

The densities of the 2 mast cell subtypes in the In IC/BPS biopsies were significantly increased compared to non-IC/BPS controls. Similarly, mast cells consistently expressed MRGPRX2, indicating their responsiveness to the neuropeptide SP. In addition, mast cell numbers and their expression of MRGPRX2 are significantly elevated in IC/BPS bladder biopsies, suggesting potential involvement in the pathophysiology of the chronic inflammation seen in the bladder walls of these patients.

CONCLUDING MESSAGE

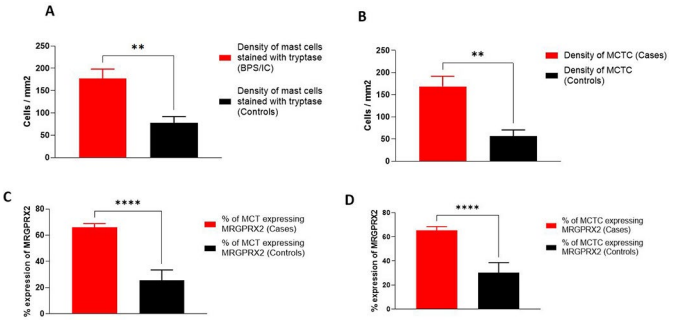
Accordingly, neurogenic inflammation caused by the SP-induced mast cell degranulation is potentially behind the chronic tissue inflammation in IC/BPS patients. Thus, blocking SP-MRGPRX2 signalling could alleviate long-standing bladder inflammation and pain in this group of patients. Moreover, other MRGPRX2 agonists including morphine, antibiotics (e.g. fluoroquinolones and vancomycin), neuromuscular blockers (e.g. Atracurium), and bradykinin B2 receptor antagonists (e.g. Icatibant), may potentially induce mast cell degranulation and subsequent bladder wall inflammation.

FIGURE 1



Each of (A, B and C) and (D, E and F) represent serial sections from one IC/BPS bladder biopsy. Arrows represent mast cell stained with antibodies for chymase (CC1), MRGPRX2 and tryptase (AA1), in sections (A and D), (B and E) and (C and F), respectively.

FIGURE 2



Graphs A, B, C and D represent the differences between IC/BPS and the control urinary bladder biopsies in the density of the mast cell subtypes MCT and MCTC (A and B, respectively) and % expression of MRGPRX2 by MCT and MCTC (C and D, respectively).

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