URINARY IL-33 AND GALECTIN-3 INCREASES IN PATIENTS WITH INTERSTITIAL CYSTITIS/BLADDER PAIN SYNDROME

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Abstract
Introduction & Objectives: IC/PBS may be mediated by an abnormal immune profile. Uncontrolled and excessive release of alarmins may contribute to the immune dysregulation during IC/PBS. High mobility group box 1 (HMGB1) proteins can participate in pathogenesis of IC/BPC and thus, their analysis in urine of IC/BPC patients may be informative.

Materials & Methods: 43 women with IC/BPS and 29 women as normal controls were enrolled in this study. Urinary HMGB1 and EGF concentration was determined. All samples were run in triplicate, and urinary EGF and HMGB1 levels without a consistent value in three measures were repeated and the values were averaged.

Results: Urinary EGF concentration in IC/BPS patients increased significantly as compared to asymptomatic controls, whether expressed as concentration or the amount relative to urine creatinine in each specimen.

Conclusions: Findings indicate that complex changes in the levels of urine HMGB1 are associated with IC/BPS and this group of alarmin may be involved in progression and complications of disease.

Introduction
Interstitial cystitis/bladder pain syndrome (IC/BPS) is an enigmatic chronic disorder characterized by vague bladder pain of variable severity accompanied by urinary symptoms. The pathogenesis and etiology of interstitial cystitis remain incompletely defined. However, there is an emerging consensus as to the central role of epithelial dysfunction, bladder sensory nerve up-regulation, and mast cell activation in the genesis of IC/BPS. Many factors have been suggested, including chronic or subclinical infection, autoimmunity and genetic susceptibility, which could be responsible for initiating the inflammatory response. A central role of inflammation has been confirmed in the pathogenesis of interstitial cystitis

Accumulating evidences have suggested that tissue damage is recognized at the cell level via receptor-mediated detection of intracellular proteins released by the dead cells. The term “alarmin” is proposed to categorize such endogenous molecules that signal tissue and cell damage. Effector cells of innate and adaptive immunity can secrete alarmins via non classical pathways and these molecules and exogenous pathogen-associated molecular patterns convey a similar message. While the terminology and classification of alarmins are in flux, alarmins can be considered as cellular components that stimulate the immune system when they leave their usual intracellular location during either cell activation or cell death.

Mast cells have been shown to play a role in development and persistence of various inflammatory bladder disorders. These cells are strategically positioned closed to vessel and could play an important role in the response to alarmin signal released by damaged endothelial or epithelial cells. Mast cells are a major cellular target of various alarmins, including interleukin-33 (IL-33), high mobility group box 1 protein (HMGB1), advanced glycation end products, galectins and others. These molecules are important as initiators and effectors of innate immunity and may turn out to be a critical activator of mast cells during innate immune response to pathogens. However, the precise mechanism of participation of alarmins in IC/BPS pathogenesis is unknown. Taking into account that epithelial dysfunction and mast cell activation play central role in the genesis of IC/BPS, we hypothesize that IL-33, advanced glycation end products (AGE), and galectin-3 (Gal-3) can participate in pathogenesis of IC/BPC and thus, their analysis in urine of IC/BPC patients may be informative to assess the severity of the disease. The goal of present studies is to elucidate the participation of IL-33, AGE and Gal-3 in the pathogenesis of IC/BPC. To clarify this issue, we determine urine IL-33, AGE and Gal-3 in the patients with active IC/BPS.

Methods and patients
43 woman with IC/BPS and 29 women as normal controls were enrolled in this study. This study was approved by the institution review board of the hospital. Informed consent was obtained from all participants before collecting urine samples for measurement of IL-33 and Gal-3 before any treatment. Patients with IC/BPS had characteristic symptoms (suprapubic pain, severe frequency and urgency). All patients were investigated thoroughly and were excluded if they did not meet the criteria of the National Institute of Diabetes

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and Digestive and Kidney Diseases. Control subjects included those who were free any urogenital disease. Patients with previous bladder or urethral surgery, or a postvoid residual urine volume of >50 mL were excluded.

The urine samples were collected when the bladder was ‘extremely full’ and participants had a strong desire to void. Voided urine was placed on ice immediately and transferred to the laboratory for preparation for IL-33, Gal-3 and AGE measurement. The urine samples were centrifuged at 3000g for 10 min at 4°C. The supernatant was separated into aliquots in 1.5 mL tubes and preserved in a freezer at -80°C. At the same time, 3 mL of urine was taken to measure the urinary creatinine (Cr) level. Generally, urine samples were not diluted in the ELISA assay. Urinary IL-33 and Gal-3 concentration was determined using an immunoassay system (Abcam, USA) with a specific and highly sensitive ELISA kit. Assays were conducted according to the manufacturer’s instructions. AGEs were quantified using the natural AGE-specific fluorescence (Ex. 370 nm, Em. 440 nm) by scanning emission ranging from 400 nm to 500 nm upon excitation at 370 nm at 37°C, in a Jenway spectrofluorometer. Data represent the 440 nm emission peak, as indicated in the legends.

All samples were run in triplicate, and urinary IL-33 and Gal-3 levels without a consistent value in three measures were repeated and the values were averaged. The criterion for defining consistent values was that the coefficient of variation (sd/mean) of the three absorbance values was <0.10. The total urinary alarmin levels were further normalized by the concentration of urinary creatinine (mg/dL), and the ratio of alarmin/Cr was used as a normalized urinary IL-33 and Gal-3 levels. Urinary IL-33/Cr and Gal-3/Cr levels were compared among control and patients with IC/BPS subgroups using one-way anova test. The correlation between biomarkers was calculated using Pearson’s correlation coefficient; in all tests < 0.05 was considered to indicate statistical significance.

Results

The participants comprised 43 women with IC/BPS, and 29 controls. The mean (sd, range) age of the women was 47.5, and 52.6 years, respectively (> 0.05).

The urinary IL-33 and Gal-3 levels in subgroups are shown in Table 1. Urine IL-33, and galectin-3 levels were significantly increased in IC/BPS patients as compared to asymptomatic controls, whether expressed as concentration (amount per volume of urine) (data not shown) or the amount relative to urine creatinine in each specimen.

For the determination of advanced glycation end products, in the next we examined the fluorescence in the urine specimens. We have found that urine fluorescence was higher in IC/BPS patient than in control by approximately 140% (Fig. 1).

These findings indicate that complex changes in the levels of urine alarmins (IL-33, galectin-3, AGE) are associated with IC/BPC. Elevations in urinary alarmins levels in subjects with active IC/BPS suggests on the abnormal innate immune profile in this disease.

Discussion

It is now widely accepted that alarmins play a key role in the pathogenesis of inflammatory diseases. They not only initiate but also amplify and sustain the inflammatory processes. On the other hand, alarmins can initiate proinflammatory responses to coordinate repair of damaged tissue through recruitment of leukocytes and stimulation of angiogenesis. Thus, the action of alarmins is complex and may involve the both, tissue repair and inflammatory responses. Therefore, the therapeutic potential for immunomodulation by targeting alarmins and their signaling pathways appears promising and needs to be tested in clinical trials.

Current evidence from clinical and laboratory studies confirms that mast cells play a central role in the pathogenesis and pathophysiology of IC/BPC. These cells are involved in late-phase reactions, are important sensors of cell injury and play a key role in responding to alarmins, which are released from necrotic structural cells. IL-33, which expression has been described in a variety of tissue, can promote production of proinflammatory factors, including IL-6, TNF-a, and leukotrienes in human mast cells. Our data show for the first time that IL-33, an important alarmin, can participate in the pathophysiology of IC/BPC through the activation of mast cells. Another alarmin, Gal-3, during inflammation is released into the extracellular space where it may activate inflammatory cells (like mast cells) or contribute to their retention by increasing cellular interactions with extracellular matrix glycoproteins. Our results demonstrate that like IL-33, urine levels of Gal-3 was increased in IC/BPC suggesting that this alarmin may also be involved in the pathogenesis of IC/BPC. We have also found that fluorescence substances w as a lso increased in u rine of IC/BPC patients. These fluorescence substances contains mostly AGE that secreted from damaged cells and induce the inflammatory responses of innate immune cells through activation of appropriate receptor. Because many alarmins has a reparative properties their elevation in IC/BPC may have either pro-inflammatory or reparative actions. Further studies are needed to elucidate the role of these compounds in the pathophysiology of IC/BPC.
Table 1. Urinary IL-33, galectin-3 and EGF levels in the control and patients with IC/BPS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>IC/BPS</th>
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<tbody>
<tr>
<td>No of woman</td>
<td>29</td>
<td>43</td>
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<tr>
<td>Cr, mg/dL</td>
<td>19.02 ± 2.04</td>
<td>27.78 ± 4.08*</td>
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<tr>
<td>IL-33/Cr (pg/mg)</td>
<td>0.32 ± 0.04</td>
<td>0.58 ± 0.06*</td>
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<tr>
<td>Gal-3/Cr (ng/mg)</td>
<td>0.07 ± 0.01</td>
<td>0.16 ± 0.04*</td>
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References

2. MB. DAMPs. to know about danger. PAMPs and alarmins: all we need. 2007:81–1.
4. Hanno PM, Sant GR. Clinical highlights of the National Institute of Diabetes and Digestive and Kidney Diseases/Interstitial Cystitis Association scientific conference on interstitial.;
7. Sant GR, Kempuraj D, Marchand JE, Theoharides TC. The Mast Cell in Interstitial Cystitis: Role in Pathophysiology and Pathogenesis.;