Brain, Behavior, and Immunity xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Toll-like Receptor 4 and comorbid pain in Interstitial Cystitis/Bladder Pain Syndrome: A Multidisciplinary Approach to the Study of Chronic Pelvic Pain research network study

Andrew Schrepf [a], Catherine S. Bradley [b,c], Michael O'Donnell [b], Yi Luo [b], Steven E. Harte [d], Karl Kreder [b,c], Susan Lutgendorf [a,b,c,*], The Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network

[a] Department of Psychology, University of Iowa, United States
[b] Department of Urology, University of Iowa, United States
[c] Department of Obstetrics and Gynecology, University of Iowa, United States
[d] Departments of Anesthesiology and Internal Medicine–Rheumatology, University of Michigan, United States

ARTICLE INFO

Article history:
Received 18 November 2014
Received in revised form 3 March 2015
Accepted 4 March 2015
Available online xxxx

Keywords:
Inflammation
Toll-like Receptors
Functional somatic syndromes
Pain
Negative affect
Interstitial Cystitis/Bladder Pain Syndrome

ABSTRACT

Background: Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a condition characterized by pelvic pain and urinary symptoms. Some IC/BPS patients have pain confined to the pelvic region, while others suffer widespread pain. Inflammatory processes have previously been linked to pelvic pain in IC/BPS, but their association with widespread pain in IC/BPS has not been characterized. *Methods:* Sixty-six women meeting criteria for IC/BPS completed self-report measures of pain as part of the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP), collected 3 days of saliva for cortisol assays, and provided blood samples. Peripheral blood mononuclear cells (PBMCs) were stimulated with Toll-like Receptor (TLR) 2 and 4 agonists and cytokines were measured in supernatant; IL-6 was also measured in plasma. Associations between inflammatory variables and the likelihood of endorsing extra-pelvic pain, or the presence of a comorbid syndrome, were tested by logistic regression and General Linear Models, respectively. A subset of patients ($n = 32$) completed Quantitative Sensory Testing. *Results:* A one standard deviation increase in TLR-4 inflammatory response was associated with a 1.59 greater likelihood of endorsing extra-pelvic pain ($p = .019$). Participants with comorbid syndromes also had higher inflammatory responses to TLR-4 stimulation in PBMCs ($p = .016$). Lower pressure pain thresholds were marginally associated with higher TLR-4 inflammatory responses ($p = .062$), and significantly associated with higher IL-6 in plasma ($p = .031$). *Conclusions:* TLR-4 inflammatory responses in PBMCs are a marker of widespread pain in IC/BPS, and should be explored in other conditions characterized by medically unexplained pain.

1. Introduction

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a highly prevalent debilitating chronic condition characterized by pelvic/bladder pain and urinary symptoms such as frequency, urgency, and nocturia (Hanno et al., 2011). Additionally, IC/BPS patients have a high prevalence of psychiatric comorbidities including depression and anxiety disorders (Clemens et al., 2008). While some patients present with Hunner's ulcers, inflammatory lesions found on the wall of the bladder, approximately 90% do not (Simon et al., 1997). IC/BPS is therefore a diagnosis of exclusion, and is sometimes considered a cluster of medically unexplained symptoms.

It has been proposed that there may be distinct subtypes of I C/BPS, as some patients appear to experience pain and discomfort in the pelvic/bladder region only (i.e. local pain), while others report extra-pelvic pain consistent with somatic syndromes like fibromyalgia, Irritable Bowel Syndrome (IBS), Chronic Fatigue Syndrome (CFS), or Temporomandibular joint disorder (TMD), suggesting a condition mediated by the central nervous system. A recent investigation found that that comorbid IBS and CFS were present in 39% and 19% of IC/BPS patients, respectively (Nickel

* Corresponding author at: E11 Seashore Hall, Iowa City, IA 52242, Unites States. Tel.: +1 319 335 2432.
E-mail address: susan-lutgendorf@uiowa.edu (S. Lutgendorf).

http://dx.doi.org/10.1016/j.bbi.2015.03.003
0889-1591/

et al., 2010). These findings are consistent with the results of many studies finding high degrees of comorbidity between somatic syndromes (Wessely et al., 1999). This suggests that there may be common physiological factors that support global changes in central pain pathways and increased pain perception (Phillips and Clauw, 2011). Furthermore, IC/BPS patients with comorbid somatic syndromes (e.g. CFS) appear to be at risk of developing additional somatic syndromes in the future, suggesting a progressive element of altered pain perception in some patients (Warren et al., 2013).

Identifying markers of pain sensitization in IC/BPS may improve early phenotyping of vulnerable patients and lead to novel therapeutic targets with the potential to prevent disease progression. Furthermore, identifying markers of central sensitization in chronic pain patients may further prevent psychiatric comorbidity as chronic pain has recently been shown to induce dysfunction in the locus coeruleus and subsequent depression and anxiety like behaviors in an animal model (Alba-Delgado et al., 2013). Much research has been devoted to identifying altered mechanisms of pain perception (i.e. sensitized pathways), and whether reliable markers of these alterations can be identified. Candidate markers include changes in pain processing networks identified through functional magnetic resonance imaging (fMRI), and hyperalgesia/allodynia identified by Quantitative Sensory Testing (QST), both of which have identified abnormal responses to stimuli in chronic pain patients, including patients with IC/BPS (Kilpatrick et al., 2014; Ness et al., 2014). Another promising biomarker is the inflammatory response to Toll-like Receptor (TLR) stimulation in peripheral immune cells, as we have recently found these responses to be associated with heightened pelvic pain in IC/BPS (Schrepf et al., 2014).

TLRs are highly conserved receptors on sentinel immune cells that respond to both Microbe Associated Molecular Patterns (MAMPs) and Damage Associated Molecular Patterns (DAMPs; Hutchinson et al., 2009). We have recently reported that TLR-2 inflammatory responses distinguish IC/BPS patients from healthy controls, and that the magnitude of TLR-4 inflammatory responses in stimulated PBMCs are associated with the extent of painful urinary and pelvic symptoms reported by IC/BPS patients. PBMCs have been hypothesized to mark pain sensitization in humans since it was demonstrated that proliferation of PBMCs incubated with morphine is strongly associated with tolerance for noxious cold stimuli (Hutchinson et al., 2004). Additionally, we found that IC/BPS patients had higher serum levels of Interleukin (IL)-6, a marker of systemic inflammation, and altered diurnal cortisol patterns (Schrepf et al., 2014). These finding echo a recent investigation that found that TLR-2 and TLR-4 inflammatory responses in PBMCs differentiate chronic pain patients from healthy controls (Kwok et al., 2012) and other work identifying altered TLR inflammatory responses as features of other conditions characterized by persistent pain such as Inflammatory Bowel Disease and Rheumatoid arthritis (Kovarik et al., 2011; Kowalski et al., 2008). However, it is unknown if inflammatory responses in PBMCs can differentiate subtypes of painful syndromes such as IC/BPS, particularly those characterized by pain not typically considered part of the IC/BPS syndrome (i.e. widespread, extra-pelvic pain).

The purpose of the current study was to determine if inflammatory processes, especially TLR-2 and TLR-4 inflammatory responses in PBMCs, are differentially associated with pelvic vs. extra-pelvic pain in IC/BPS. Additionally, we examined relationships between TLR-mediated inflammation, pain intensity/interference with daily life, and pressure pain sensitivity determined by QST. We also examined the relationship between TLR-mediated inflammation and the presence of comorbid pain conditions in IC/BPS patients, as these conditions are characterized by pain outside the pelvic region, and contribute substantially to the difficulty of treating the IC/BPS syndrome (Nickel et al., 2010). In line with the results of our earlier work and results from animal models of chronic pain, we hypothesized that greater TLR inflammatory responses in PBMCs would be associated with pain outside the pelvic region, increased pain sensitivity by QST, and comorbid somatic syndromes.

2. Methods

2.1. MAPP study and recruitment

The Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) is a National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) sponsored research initiative comprising several sites with the objective of characterizing the epidemiology, symptom trajectories, phenotypes and biological correlates of chronic pelvic pain (Clemens et al., 2014). The University of Iowa is a participating institution emphasizing biomarker research. Participants were eligible if they were at least 18 years of age, female, not pregnant, and reported chronic pain/pressure/discomfort associated with the bladder or pelvic region in the preceding three months. Participants had negative urine cultures for uropathogens. Exclusion criteria included conditions which might result in tissue damage to areas relevant to IC/BPS symptomology (e.g. history of urethral stricture, neurological disorder affecting the bladder or bowel). Additional information about the MAPP project, including patient characterization, study aims, and full exclusion criteria is available (Clemens et al., 2014; Landis et al., 2014; Schrepf et al., 2014).

2.2. Demographic and symptom information

The sample was composed of an expanded group of participants from a previously reported study (Schrepf et al., 2014) who provided additional information on pain and comorbid syndromes. In addition, a subsample completed QST. Sixty-six women provided demographic information at the time of eligibility screening, including information about income, education, employment, race and ethnicity. Upon study entry, participants had a blood draw, urine collection, physical examination and completed a battery of questionnaires relating to pain and urological symptoms. These included the Brief Pain Inventory (BPI), a measure of pain intensity, interference with daily life, and a body map for selection of painful areas (Cleeland and Ryan, 1994) which has previously been validated in chronic pain populations (Tan et al., 2004). The body map was modified so that patients could select regions where pain was experienced from a standardized form of 45 distinct areas. Participants were also administered self-report screens to assess the presence of comorbid somatic syndromes. These included the Rome III criteria for IBS (Drossman and Dumitrascu, 2006), the American College of Rheumatology diagnostic criteria for Fibromyalgia (Wolfe et al., 2010), International Chronic Fatigue Syndrome Study Group criteria for CFS (Fukuda et al., 1994), an 8 question MAPP specific diagnostic tool for symptoms of vulvodynia (e.g. "experience constant burning or raw feeling at the opening of the vagina,") and the Research Diagnostic Criteria for TMD (Dworkin et al., 2002). These diagnostic criteria show adequate reliability and validity (Dworkin et al., 2002; Ford et al., 2013; Komaroff et al., 1996; Wolfe et al., 2010) excepting the criteria for vulvodynia, which is necessarily exploratory. Additionally, participants completed the reliable and validated Positive and Negative Affect Scale (PANAS; Watson et al., 1988). Use of non-steroidal anti-inflammatory drugs (NSAIDs), tricyclic antidepressants, opioids, selective serotonin/norepinephrine reuptake inhibitors (SSRI/SNRI), and pentosan polysulfate, and duration of symptoms in years, were collected by patient self-report.

Please cite this article in press as: Schrepf, A., et al. Toll-like Receptor 4 and comorbid pain in Interstitial Cystitis/Bladder Pain Syndrome: A Multidisciplinary Approach to the Study of Chronic Pelvic Pain research network study. Brain Behav. Immun. (2015), http://dx.doi.org/10.1016/j.bbi.2015.03.003