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Review Article

Pathophysiology of interstitial cystitis/bladder pain syndrome

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ABSTRACT

Interstitial cystitis (IC) is a heterogeneous syndrome characterized by bladder pain and is associated with frequency and nocturia. Research findings have proposed several pathophysiological mechanisms including epithelial dysfunction, activation of mast cells, neurogenic inflammation, autoimmunity, and occult infection. One of the most common findings in IC bladders is denudation or thinning of the bladder epithelium, suggesting an altered regulation of urothelial homeostasis. Different phenotypes of IC have been explored including Hunner and nonHunner type IC (ulcer and nonulcer type IC), hypersensitive bladder, and bladder pain with and without functional somatic syndrome. Different gene expressions have been found in different IC phenotypes. Augmented purinergic signaling in the bladder has been found in IC. Significant increases in antiproliferative factor, decreases in heparin-binding epidermal growth factor, and increased levels of epidermal growth factor have been discovered. Abnormal cytokine secretion in IC bladders has also been related to an increase in purinergic signaling, which mediates increased bladder sensation. Abnormal expression of uroplakins, chondroitin sulfate, junction protein E-cadherin, and tight junctional protein zonula occludens-1, strongly suggests abnormal epithelial differentiation in IC bladders. The increased apoptosis of urothelium has been associated with increased tryptase activity and clinical bladder pain scores, suggesting chronic inflammation, increased apoptosis, and abnormal urothelial function are closely associated in IC bladders. A local inflammatory process might be induced through the afferent and efferent nerves in the suburothelial interstitial cellular network, which integrates the transmission of signals from the urothelium to the detrusor muscles in the bladder wall. These urothelial dysfunctions can be partially reversed after repeated bladder injections of botulinum toxin A. Multiple comorbidities and functional somatic syndromes are also found to associate with IC, together with increased sympathetic nervous system tonicity and increased serum proinflammatory proteins and cytokines. Thus, the pathogenesis of IC might be involved in systemic disorders. It is possible to postulate that the pathophysiology of IC might evolve sequentially by: (1) urothelial injury (urinary tract infection, surgical trauma, chronic bladder overdistention); (2) suburothelial inflammation; (3) chronic inflammatory cell infiltration in the suburothelium; and (4) increased inflammatory reaction in the sensory afferents, dorsal horn ganglia, and corresponding spinal cord. IC might be considered a progressive disease that evolves from early stage to late stage bladder conditions. Insult to the visceral organ initiates an inflammatory process, causing urothelial dysfunction in the bladder. The inflammatory reaction proceeds along the sensory nerves in the dorsal root ganglion as well as the sacral cord. The sensory impulses also ascend to the corresponding cortical gyrus. Patients might have an early inflammatory reaction and produce characteristic IC symptoms, including bladder pain, urgency, frequency, and a positive potassium chloride sensitivity test. If the insult does not continue, the inflammation resolves and patients may have symptom relief after symptomatic treatment. However, if the bladder insult continues, the inflammatory reaction increases to a higher level, causing permanent inflammation printing.

Conflict of interest: none.

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1. Introduction

Interstitial cystitis (IC) is characterized by bladder pain, frequency, and nocturia. There is no clear definition of IC as the clinical symptoms and histology are not specific. The diagnosis of IC can only be made based on a combination of a thorough patient history, sterile and cytologically negative urine, cystoscopic hydrodistention under anesthesia, and bladder biopsy. Many different etiologies have been proposed including: (1) a postinfection autoimmune process; (2) mast cell activation induced by inflammation, toxins, or stress; (3) urothelial dysfunction and increased permeability of the urothelium; and (4) neurogenic inflammation. However, none of these etiologies has been definitely proven [1]. This review highlights recent research findings in the pathophysiology of IC/bladder pain syndrome (BPS).

2. Urothelial barrier dysfunction

Recent findings have proposed several pathophysiological mechanisms for IC, including epithelial dysfunction, activation of mast cells, neurogenic inflammation, autoimmunity, and occult infection [2]. One of the most common findings in bladder mucosal biopsies from IC patients is denudation or thinning of the bladder epithelium, suggesting an altered regulation of urothelial homeostasis [3].

The urothelium of the urinary bladder acts as a barrier between urine and its solutes and the underlying bladder. Bladder surface mucus is a critical component of this function [4–6]. Glycosaminoglycans (GAG) are extremely hydrophilic and trap water at the outer layer of the umbrella cell. In patients with IC, disruption of the urothelial barrier may initiate a cascade of events in the bladder, leading to symptoms and disease. Urothelial barrier dysfunction leads to the migration of urinary solutes, in particular, potassium, which depolarizes nerves and muscles and causes tissue injury [7,8].

The urothelium plays an important role in communicating with bladder nerves, smooth muscle, immune cells, and inflammatory systems [5]. A leaky epithelium has been considered the primary disease that causes urinary potassium leakage into the suburothelium and generates symptoms of frequency urgency and bladder pain [9,10]. Intravesical potassium chloride instillation at a concentration of 0.4M was found to provoke symptoms in 4.5% of healthy people, 70% of patients with IC, 18% of IC patients treated with heparin, and 100% of patients with irradiation cystitis [6]. A positive potassium stimulation test causing an increase in bladder pain of ≥ 2 on a visual analogue scale and a small cystometric capacity were found to be 100% predictive of IC in patients with hypersensitive bladder syndrome [11]. Intravesical sulfated polysaccharide was found to restore injured urothelium to normal [12]. A recent study demonstrated that intravesical GAG replenishment therapy also produces a physiological effect of decreasing recruitment of inflammatory cells in an acute damaged bladder rat model [13].

A subset of patients with IC might have a failure of urothelial cytodifferentiation, which might contribute to the disease and bladder dysfunction [4]. Abnormal expression of molecular markers has been found in IC bladder biopsies. Abnormal expression of uroplakins, chondroitin sulfate, and tight junctional protein zonula occludens-1 (ZO-1) strongly suggests abnormal differentiation in bladders with IC, whereas elevated E-cadherin expression may represent an adaptation to increased bladder permeability [14].

Recent studies have shown increased apoptosis of the urothelium in patients with IC. Terminal deoxynucleotidyl transferase dUTP nick end labeling staining showed apoptotic cells in the microvascular endothelial cells but not in the endothelial cells of venules in IC bladders [15]. Higher levels of urothelial cell apoptosis and abnormal E-cadherin expression in IC bladders were associated with chronic inflammation [16]. Bladder epithelial cells from patients with IC exhibited profoundly decreased proliferation [16]. The proliferation rate of explanted bladder epithelial cells from patients with IC was significantly decreased compared to that of control cells, indicating an intrinsic abnormality in IC cell proliferation. This abnormality may be caused by an antiproliferative factor (APF), which induces reversible inhibition of heparin-binding epidermal growth factor-like (HB-EGF-like) growth factor production and normal bladder epithelial cell proliferation [17]. APF treatment caused significant increases in the paracellular permeability of normal bladder epithelial cell monolayers and the attenuation of tight junctions compared with mock APF, similar to changes seen in IC cells. APF treatment also decreased expression of the tight junction proteins ZO-1 and occludin [18,19]. These results are consistent with previous reports. In patients with IC, increased levels of cleaved caspase-3, Fas, and cleaved caspase-8 were found, suggesting activation of the extrinsic apoptotic pathway [20].

3. Chronic inflammation of the suburothelium in IC

Most urologists consider IC a result of long-standing inflammation of the bladder [3]. Bladder histological analysis shows infiltrates of mast cells, eosinophilic leukocytes, and T-lymphocytes, suggesting IC is mediated by the immune system [21]. Biopsies of IC bladders have confirmed the involvement of eosinophils, macrophages in the urothelium, and mast cells in the detrusor. Involvement of eosinophils is also supported by urine cytology showing increased urinary eosinophil cationic protein in the urine [22,23]. Our previous study found increased serum immunoglobulin E levels in patients with ketamine cystitis [24]. However, the participation of immunoglobulin E-mediated inflammation in the pathogenesis of the bladder inflammation in IC has not been investigated. Mast cells have been considered crucial effector cells for the immune response implicated in the pathogenesis of IC [21]. High expression of T- and B-cell markers, low expression of urothelial markers, focal lymphoid aggregates in the submucosa, and high immunoglobulin concentrations in the urine were found in patients with IC European Society for the Study of Interstitial Cystitis type 3C [25]. A recent study showed that suburothelial mast cell distribution was characteristic of IC with Hunner's lesions, whereas detrusor mastocytosis had a poor predictive value for IC [26].

Chronic suburothelial inflammation might inhibit normal basal cell proliferation and affect apical urothelial function. Treatment of urothelial dysfunction cannot be based solely on replacement of defense glycoproteins in the bladder urothelium. Furthermore, bladder inflammation caused by intravesical irritants or that in patients with IC leads to acute afferent nerve activity and long-term plasticity that lowers the threshold for nociceptive and mechanoceptive afferent fibers [27,28]. A rise in bladder nerve growth factor (NGF) in the muscle or urothelium initiates signals that are transported along the afferent nerves of the bladder to the dorsal root ganglion or spinal cord [29,30].

Previous studies showed that intravesical injection of botulinum toxin A (BoNT-A) reduced bladder pain in patients with refractory IC [31,32]. NGF mRNA levels in the bladder tissue significantly increased in patients with IC and decreased to the normal range after BoNT-A treatment [33]. Repeated BoNT-A injections plus hydrodistention might provide a better outcome in treating IC compared with hydrodistention alone. Urothelial apoptosis and E-

cadherin expression improved in IC patients who responded to repeated BoNT-A injections, and this was associated with a reduction in bladder pain scores [34]. Chronic suburothelial inflammation might alter urothelial function and cell differentiation, and BoNT-A injections might reduce inflammation and restore a healthy urothelium, thereby improving the clinical symptoms of IC.

Previously published results confirm that IC involves an aberrant differentiation program in the bladder urothelium that leads to altered synthesis of several proteoglycans, cell adhesion and tight junction proteins, and bacterial defense molecules such as GP51. Therefore, replacement therapy with GAG has been widely used for treatment of IC [35]. However, further correlation of the expression of the proteoglycan core proteins and differentiation-related markers with inflammation scores in IC bladder biopsies revealed that the abnormalities in urothelial differentiation and loss of barrier function in IC were independent of inflammation [22].

4. Glomerulations in the bladder urothelium in IC

In previous reports, histological evidence showed several marked changes in various tissue elements. Firstly, abnormal behavior of urothelial cells disrupted the permeability barrier. Secondly, the vascular lesions included endothelial cell injury and suggested a slow microcirculation. Thirdly, neural changes included a combination of degenerative and regenerative features [36,37].

Several important proteins associated with bladder inflammation were associated with increased angiogenesis and glomerulations in IC. Glomerulations during cystoscopic hydrodistention were highly associated with overexpression of angiogenic growth factors such as platelet derived endothelial cell growth factor/thymidine phosphorylase [38], vascular endothelial growth factor (VEGF) [36], and hypoxia-inducible factor-1-alpha [39]. Measuring the urine concentrations of these angiogenic growth factors may be a new and useful method for the diagnosis of IC.

VEGF plays a key role in bladder inflammation and is closely associated with the vascular alterations observed in patients with IC. Increased VEGF was associated with bladder inflammation and a small functional bladder capacity in IC patients. VEGF decreased after repeated BoNT-A injections [40].

5. Neurogenic inflammation in IC/PBS and central nervous sensitization

There is increasing evidence for the role of neurogenic inflammation in the pathophysiology of several diseases, including asthma, arthritis, migraine, and, possibly, IC [41]. Preliminary studies have shown an increase in levels of immunoreactive substance P and NGF in the bladder tissue and urine [42,43]. P2X3 receptors have also been shown to localize on the suburothelial C-fibers and detrusor muscles, and colocalize with other sensory receptors, such as transient receptor potential vanilloid receptors, euokinin receptor 1, calcitonin-gene receptor protein, tyrosin kinase A, and other sensory-related receptors [44]. IC was found to associate with increased urinary adenosine triphosphate (ATP) and increased stretch-activated ATP release by bladder urothelial cells, suggesting augmented purinergic signaling in the bladder [45]. Any insult to the urothelium or directly to the bladder wall may induce a cascade of inflammatory reactions and produce painful inflammation, such as in IC [46].

The chronic pain symptomatology in IC may also be due to central nervous system sensitization and persisting abnormality or activation of the afferent sensory system in the urinary bladder [47]. Increased central c-fos expression has been demonstrated in animal models of neurogenic detrusor overactivity and chronic inflammation. Elimination of rat spinal neurons expressing euokinin receptor 1 receptors reduced bladder overactivity and spinal c-fos expression induced by bladder irritation [48,49]. If the neurogenic inflammation in the dorsal root ganglia can be eliminated gradually through intravesical treatment, the visceral pain in IC can thus be relieved.

The afferent system in the bladder plays an important role in lower urinary tract dysfunction [50]. The symptoms of sensory urgency were associated with the increased expression of transient receptor potential vanilloid receptors mRNA in the trigone mucosa [51]. There was a significantly greater release of ATP following mechanical stretch of the urothelium from IC bladders [52]. Mechanical stimulus in IC patients induced a significantly higher stimulus-response curve, and a segmental hyperalgesia was noted, suggesting spinal central sensitization was involved in IC pathophysiology [53].

There is increasing evidence showing that afferent hyperexcitability is a result of neurogenic bladder inflammation [54]. Overexpression of NGF in mouse urothelium leads to neuronal hyperinnervation, referred somatic pelvic sensitivity, elevated mast cells, and changes in bladder function [55]. Women with bladder oversensitivity in the absence of urinary tract infection (UTI) had a significantly lower median voided volumes at a strong desire to void and maximal cystometric capacity than controls, suggesting an increased hypersensitivity [56]. Increased urothelial cell apoptosis, increased mast cell counts, and lower junctional protein E-cadherin were noted in women with recurrent UTI. These urothelial dysfunctions might explain the hypersensitivity symptoms in women after recurrent UTI [57]. A recent study suggested IC might be mediated by factors including changes in the properties of peripheral bladder afferent pathways responding to normally innocuous stimuli [58].

6. Alteration of urothelial factors and cytokines in IC/BPS bladders

APF is a small glycoprotein made specifically by bladder epithelial cells in patients with IC which induces changes in expression of certain epithelial cell proteins and profoundly inhibits cell growth. APF may affect the increased permeability and decreased tight junction formation of bladder epithelial cells and contribute to the urothelial leak and bladder pain symptoms seen in IC [18,59]. Significant increases in APF, decreases in HB-EGF, and increased levels of EGF were discovered in the urine samples of patients with IC [60]. Microarray analysis indicated that APF can also induce changes in the pattern of cellular gene expression toward a more differentiated phenotype [60]. Previous results indicate that APF treatment causes significant increases in the paracellular permeability of normal bladder epithelial cell monolayers and the attenuation of tight junctions compared with mock APF, similar to changes seen in IC cells. APF may contribute to the leakiness of the bladder epithelial barrier seen in IC [61]. Antagonist of APF significantly increased mRNA expression of ZO-1, occluding, and claudin 1, claudin 4, claudin 8, and claudin 12 in IC cells, normalized epithelial cells, and decreased paracellular permeability [62].

The human bladder urothelium and urothelial cells play important roles in the normal defense mechanism. APF expressed by the urothelial cells induces increased permeability in cell cultures, and regulates expression of other cytokines, including upregulation of HB-EGF and down-regulation of EGF. These cytokine abnormalities are also related to increases in purinergic signaling, which mediates increased bladder sensation. Alterations of uroplakins, glycoproteins expressed only in the apical urothelial cells, may result in bladder symptoms related to increased permeability or decreased protective function [63]. The abnormal

differentiation in IC urothelium with a loss of barrier proteoglycan core proteins is independent of inflammation, further suggesting different subtypes of IC [21].

7. Urinary nerve growth factor and inflammatory cytokines in IC/BPS

IC involves an aberrant differentiation program in the bladder urothelium that leads to altered synthesis of several proteoglycans, cell adhesion, tight junction proteins, and bacterial defense molecules such as GP51. These findings led to the rationale for searching for potential clusters of urinary biomarkers to detect IC in patients with overactive bladder (OAB) and frequency urgency syndrome [64].

In the urinary tract, NGF is produced by bladder smooth muscles and the urothelium [65]. NGF is also involved in ongoing regulation of neural function in conditions such as spinal cord injury and denervation, as well as in inflammation and pain [2,65,66]. Increased levels of NGF were also reported in the bladder tissue and urine of patients with sensory urgency and IC [43,67]. Intravesical injections of BoNT-A reduced bladder pain in patients with refractory IC [33]. NGF levels decreased to the normal range after BoNT-A treatment [68]. However, urine NGF is also increased in several lower urinary tract conditions such as UTI, bladder outlet obstruction, and urinary tract stones [67]. Urinary NGF levels are closely related to visual analog scores for pain and response to conventional treatment for IC [69,70]. A decrease in the urinary NGF level was associated with greater pain reduction and a successful response to treatment, suggesting that urinary NGF levels could be a useful biomarker for the severity of the bladder condition in patients with IC [69].

Urinary inflammatory mediators have been investigated as noninvasive markers to identify IC [70]. Interleukin (IL)-6 levels increased in the urine samples of IC patients and were positively associated with pain scores [71]. Nonetheless, IL-6 was only detected in the urine samples of patients with severe IC, but not those with mild IC [72]. Both IL-6 and IL-8 levels were elevated in patients with active IC [73], but similar levels were found in patients who met or did not meet the National Institute of Diabetes and Digestive and Kidney Disorders criteria for IC [74]. IL-8 is an important normal urothelial growth factor and is necessary for normal urothelial cell survival *in vivo* and *in vitro*. Lower IL-8 expression levels contribute to the pathophysiology of IC [75]. A combination of several particular elevated cytokines in the urine raises the diagnostic rate for IC and might differentiate IC from noninflammatory bladder conditions.

8. Systemic involvement in the pathogenesis of IC/BPS

Chronic inflammation is implicated in the development of OAB and IC. Elevation of serum C-reactive protein (CRP) levels is associated with chronic inflammation and lower urinary tract symptoms. Serum CRP levels were significantly higher in patients with OAB and IC than in control individuals [76]. Nevertheless, serum CRP levels could be used to identify IC in patients with irritating bladder symptoms and those refractory to antimuscarinic therapy.

Serum NGF was also found to be elevated in IC patients. The mean serum NGF level was higher in patients with IC than control individuals. No significant correlation was found between the serum and urinary NGF levels in IC patients. The clinical characteristics and medical comorbidities did not show significant differences between IC patients with high and low serum NGF levels [77]. These findings suggest that IC might be a heterogeneous syndrome involving bladder-confined inflammation or systemic inflammation.

Cytokines and chemokines play crucial roles in the pathogenesis of several chronic inflammatory diseases. The upregulated profile of serum IL-1β, IL-6, tumor necrosis factor-α (TNF-α), and IL-8 in IC patients could potentially have a prognostic role and/or serve as a tool in choosing a proper therapeutic agent for treatment. Serum proinflammatory cytokine (IL-1β, IL-6, and TNF-α) and chemokine (IL-8) levels were significantly higher in the sera of patients with IC than controls [78]. A significant correlation was found between IL-1β and IL-8, IL-6 and CRP, IL-6 and IL-8, and IL-6 and TNF-α in serum samples from IC/BPS patients. Increased expression of proinflammatory cytokine (IL-1β, IL-6, and TNF-α) and chemokine (IL-8) levels in the serum of IC patients implies not only mast cell activation, but also the possible important roles of some other inflammatory mediators in the pathogenesis of IC.

Nickel et al [79] recently proposed a phenotyping system, called UPOINT, to classify women with IC according to clinically relevant urinary, psychosocial, organ specific, infection, neurological/systemic, and tenderness domains. Increased symptom duration led to a greater number of domains, and domains that functioned outside of the bladder predicted a significant impact on symptoms. IC patients reported more pain than controls in all reported body areas. The increased pain phenotype was associated with poorer psychosocial adjustment and diminished physical quality of life [80].

IC patients have higher odds of comorbid neurological diseases, rheumatological diseases, and mental illnesses [81]. Patients with functional somatic syndrome are at risk of IC [82]. Functional somatic syndromes are risk factors for hysterectomy in patients with early symptoms of IC [83]. A distinct phenotype of patients with IC and associated multiple sensitivities with confirmed allergies/sensitivities to medications and environmental factors has been identified [84]. Myofascial pain was demonstrated in 78.3% of IC patients with at least one trigger point, and 67.9% of patients with numerous trigger points [85]. Patients with the “pelvic pain beyond” phenotype reported a higher prevalence of irritable bowel syndrome and fibromyalgia, and more general fatigue and psychiatric conditions [86].

Sympathetic nervous system dysfunction is common in IC [87]. Noradrenaline levels in the blood at resting conditions and in 24-hour urine samples were significantly higher in IC/BPS patients [88]. IC patients had diminished vagal activity and a shift towards sympathetic nervous system dominance [89]. IC patients with typical cystoscopic findings showed a marked autonomic response (increase in heart rate, systolic and diastolic blood pressure) [90].

Different phenotypes of IC have been noted. IC European Society for the Study of Interstitial Cystitis type 3C accounted for 55% of cases. Patients with nonHunner disease were on average 20 years younger, and had a significantly larger maximum bladder capacity than those with Hunner's disease [91]. Significantly different molecular characteristics were found in IC patients with lower bladder capacities [92].

DNA microarray analysis followed by quantitative real-time polymerase chain reaction revealed overexpression of genes related to immune and inflammatory responses in IC patients, including T-helper type 1 related chemokines, and cytokines such as *CXCR3* binding chemokines, and TNF (ligand) superfamily, member 14 (*TNFSF14*). These genes are potential biomarkers for IC [93]. Gene expression analysis of urine sediment found that patients with Hunner lesions had increased proinflammatory gene expression similar to that in a prior microarray bladder biopsy study. However, the analysis failed to discriminate between patients with nonHunner IC and control individuals [94]. It is possible to use quantitative proteomics and microarray analysis to identify genes that are potentially regulated by APF with a low false discovery rate. This molecular signature reflects the biological processes of cell adhesion, cell proliferation, and inflammation, constituting the pathophysiology of IC [95].

9. Conclusion

From the above evidence, it is possible to postulate that the pathophysiology of IC syndrome might evolve sequentially by: (1) urothelial injury (UTI, surgical trauma, chronic overdistention); (2) suburothelial inflammation; (3) chronic inflammatory cell infiltration in the suburothelium; and (4) increased inflammatory reaction in the sensory afferents, dorsal horn ganglia, and corresponding spinal cord.

IC might be considered a progressive disease that evolves from early stage to late stage bladder conditions. Insult to the visceral organ initiates an inflammatory process causing urothelial dysfunction in the bladder. The inflammatory reaction proceeds along the sensory nerves in the dorsal root ganglion as well as the sacral cord. The sensory impulse also ascends to the corresponding cortical gyrus. Patients might have an early inflammatory reaction and produce characteristic symptoms of IC, including bladder pain, urgency, frequency, and a positive potassium chloride sensitivity test. If the insult does not continue, the inflammation resolves and patients may have symptom relief after symptomatic treatment. However, if the bladder insult continues, the inflammatory reaction increases to a higher level, causing permanent inflammation printing.

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