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The Role of Viral Infection in the Pathogenesis of Interstitial Cystitis/Bladder Pain Syndrome

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Abstract

Purpose of Review The pathogenesis of interstitial cystitis/bladder pain syndrome (IC/BPS) is still unclear. The diagnosis of IC/BPS is made after ruling out bacterial cystitis. However, viral infection in the bladder might be a pathogenic factor for IC/BPS. The purpose of this review is to demonstrate the association between viral infection and IC/BPS.

Recent Findings The presence of urinary tract viruses in patients with IC/BPS has been sporadically investigated since the 1970s. Early studies used viral culture to investigate urine and bladder tissue samples from patients with IC/BPS, but viruses were rarely detected. With polymerase chain reaction, several studies have reported increased papillomavirus, BK virus, and John Cunningham virus viral load in urine samples from patients with IC/BPS. The presence of urinary papillomavirus was associated with more severe IC/BPS symptoms. Recent studies have used transcriptomic RNA sequencing to investigate gene expression in bladder tissue samples from patients with IC/BPS. Enriched viral infection-associated gene pathways in patients with IC/BPS were noted in the studies, including cytomegalovirus infection, Kaposi sarcoma-associated herpesvirus infection, human papillomavirus infection, and Epstein–Barr virus (EBV) infection. Our recent studies reported the presence of EBV in IC/BPS bladders, especially in patients with IC/BPS with Hunner's lesion (HIC). EBV latency and lytic infection were observed in HIC bladders, indicating EBV infection persistence and reactivation. EBV latency infection in B cells could induce brain-derived neurotrophic factor overexpression and might cause nerve hyperplasia in IC/BPS bladders.

Summary The presence of urinary virus in the patients with IC/BPS suggested that viral infection might be a pathogenic factor in patients with IC/BPS. Molecular evidence from IC/BPS bladder tissue also showed that viral infection may involve the pathogenesis of IC/BPS. Further studies are needed to clarify the mechanism.

Keywords Interstitial cystitis · Virus · Pathogenesis · Etiology · Infection

Introduction

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a highly heterogeneous syndrome characterized by persistent suprapubic/bladder pain and lower urinary tract symptoms, including urinary frequency or urgency [1]. A recent study has revealed that the estimated prevalence of IC/BPS according to high sensitivity and specificity definitions is 4.2% and 1.9%, respectively [2]. In the past century, investigators have put great effort into understanding the pathogenesis of this disease. However, the detailed mechanisms remain a mystery. Several important pathophysiological mechanisms in IC/BPS have been reported, including autoimmunity, past urinary tract infection, bladder epithelial barrier dysfunction, central nerve system sensitization, neurogenic inflammation, and sensory receptor dysregulation [3–5]. However, none of them could well explain the pathophysiology of IC/BPS. The actual pathogenesis of IC/BPS is still highly debatable. Since no definite pathogenesis of IC/BPS has yet been elucidated, most treatments of IC/BPS focus on symptom relief rather than curing the disease. Current guidelines recommend using oral pentosan polysulfate sodium, intravesical hyaluronic acid installation, or intravesical botulinum toxin injection for treating patients with chronic IC/BPS [1]. Although these treatments provide symptom relief for patients with IC/BPS, they rarely cure the disease, and symptom relapse is expected after treatment.

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Currently, the diagnosis of IC/BPS is made by ruling out bladder bacterial infection, which can be confirmed by urinary analysis and urine culture [1]. However, other pathogens may cause IC-like symptoms and make the diagnosis of IC/BPS confusable. The National Institute of Diabetes, Digestive and Kidney Diseases criteria recommend ruling out tuberculosis cystitis and herpes infection before making a diagnosis of IC/BPS [6]. Human papillomavirus, *Chlamydia trachomatis*, and mycoplasma infection can also be considered possible differential diagnoses of IC/BPS [7]. However, tests of these possible pathogens are rarely performed for patients with IC/BPS in clinical practice, and the association between microorganism infection in the bladder and the pathogenesis of IC/BPS is still uncertain. Histopathology studies of the bladders of patients with IC/BPS have shown lymphocyte infiltration and aggravation in the lamina propria [8•], which is consistent with virus infection in mucosal tissue [9•]. Since the 1970s, virus infection in the bladder, a possible pathogenesis of IC/BPS, has been investigated [10]. Previous studies have reported the presence of adenovirus, John Cunningham virus (JCV), BK virus (BKV), or herpes simplex virus (HSV) in patients with IC/BPS [10–12, 13•, 14, 15]. Furthermore, recent studies have reported the presence of Epstein–Barr virus (EBV) in the bladders of patients with IC/BPS [16••] and provided molecular evidence to support the idea that EBV infection may be involved in the pathogenesis of IC/BPS [17••]. Virus infection in the bladder might be a cause of IC/BPS. Thus, this study aimed to review the current evidence on virus infection in patients with IC/BPS and the role of viral infection in the pathogenesis of IC/BPS.

Evidence of Urinary Tract Virus in Patients with IC/BPS

The methods used to detect viruses in human samples have been developed over time. Viral culture is the gold standard for detecting an active-replicative virus and assessing its infectious potential [18, 19]. In 1970, Hanash et al. first reported their results of urine viral culture for patients with IC/BPS [10]. Rhesus monkey kidney cells, HeLa cells, and human diploid fibroblasts were inoculated with buffered urine and observed for cytopathic effects or hemadsorption (indications of viral activity) for 10–14 days. However, no virus was isolated from the urine of patients with IC/BPS. In 1985, Fall et al. studied 41 patients with IC/BPS and used viral culture and enzyme-linked immunosorbent assay for HSV to detect viruses in urine samples from the patients [11]. The results were negative. Additionally, Keay et al. used urine and bladder biopsy samples to culture the virus, and cytomegalovirus (CMV) was isolated from one urine sample [12]. The results showed that all bladder samples were negative for the virus. Using viral culture to identify viruses in human samples might be the gold standard for defining viral infections in human organs. However, the sensitivity of viral culture remains highly controversial [20]. Additionally, viral culture is limited by the type of cell lines that can be used to grow viruses. Nowadays, immunochemical staining, nucleic acid hybridization, and real-time quantitative polymerase chain reaction (RT-qPCR) are more widely used to detect viruses in human samples, especially in clinical practice [20].

RT-qPCR for urine samples has been widely used to diagnose urinary tract BKV or CMV infection in immunocompromised patients with hemorrhagic cystitis [21, 22]. Previous studies used PCR for bladder biopsy specimens from patients with IC/BPS to identify CMV, adenovirus, HSV types I and II, and human papillomavirus [13•, 23]. However, all specimens were negative for these viruses. In 2009, Eisen et al. reported two patients with IC/BPS who had high urinary levels of polyomavirus, detected by RT-PCR [24•]. One of the two patients had IC/BPS with Hunner's lesion (HIC) and received intravesical instillation of cidofovir weekly for 5 weeks. The patient showed significant improvement in bladder pain and urinary frequency after the intravesical antiviral treatment, and the urinary viral load significantly decreased. After that, polyomavirus as a potential causative etiology of IC/BPS attracted the attention of researchers. In 2014, Ridder et al. reported positive BKV titers, quantitatively determined by RT-PCR, in urine samples from 11 of 15 patients with IC/BPS, while the urine samples from the 8 control subjects were negative [25]. In 2015, Winter et al. investigated 50 patients with IC/BPS and age-matched controls for polyomaviruria [14]. Polyomaviruses, BKV and JCV, were detected in urine and bladder samples by RT-PCR. Polyomavirus urinary shedding was more prevalent in patients with IC/BPS than in controls (56% vs. 32%, $p = 0.02$). PCR testing revealed that only one of 19 bladder tissue samples from patients with IC/BPS was polyomavirus DNA positive, and all 19 bladder tissue samples were negative in polyomavirus immunohistochemistry tests. Notably, urinary polyomavirus, particularly BK viruria, was significantly associated with bladder ulceration. Recently, the Multidisciplinary Approach to the Study of Chronic Pelvic Pain Research Network has performed next-generation sequencing to look for the presence of viruses in urine [15]. The presence of polyomavirus DNA and several RNA viruses, such as human immunodeficiency virus and anelloviruses, was investigated in the pooled urine samples, and only polyomavirus DNA was detected. Further analysis revealed that patients with urinary polyomaviruses had more severe bladder pain and urinary frequency. Additionally, using high-throughput sequencing technology, polyomaviruses were identified in 95% of urine samples from patients with IC/BPS and 0% from the control group.

Several studies have detected urinary polyomaviruses, such as JCV and BKV, in patients with IC/BPS. However, evidence showed that the presence of viruses in IC/BPS bladder tissue was still rare. Additionally, BK viruria is common in patients with renal transplant dysfunction [26]. However, bladder pain is rarely reported among renal transplant patients with BK viruria. The role of polyomavirus bladder infection in the IC/BPS pathogenesis mechanism is still uncertain.

Evidence of Virus Infection Involves the Pathogenesis Mechanism of IC/BPS

Suburothelial inflammation and urothelium denudation are the most well-known histopathological characteristics of IC/BPS bladders [27]. Specifically, inflammation in IC/BPS is characterized by lymphoplasmacytic cell and mast cell infiltration [27, 28]. Viral infections in mucosal tissues exhibited eroded epithelium and lymphoplasmacytic infiltration in the lamina propria [9•]. Previous studies have reported upregulated inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor-α (TNF-α), in IC/BPS bladders [29, 30••]. However, few studies have investigated their origin. Similarly, IL-1, IL-6, and TNF-α are the most important inflammatory cytokines during viral infection [31, 32]. Recently, several studies of RNA sequencing of bladder mucosal biopsies have provided indirect evidence to support the idea that virus infection may involve the pathogenesis of IC/BPS [30••, 33, 34]. In 2019, Akiyama et al. first reported the whole transcriptome profiling RNA sequencing data of human IC/BPS bladder samples [30••]. They identified 112 upregulated Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in HIC bladders. In their study, the human CMV infection, Kaposi sarcoma-associated herpesvirus infection, human papillomavirus infection, and EBV infection KEGG pathway were ranked among the first 15 upregulated pathways. Saha et al. analyzed 4 Gene Expression Omnibus datasets and revealed 51 upregulated differentially expressed genes (DEGs) in IC/BPS [33]. They found upregulated EBV infection and viral myocarditis KEGG pathways. More recently, Peng et al. have investigated immune cells in IC/BPS bladders using single-cell RNA sequencing [34]. They reported significantly increased regulatory T cells with enriched virus receptor activity and viral transcription DEGs in IC/BPS bladders. The above molecular evidence indirectly supported the idea that viral infection in the bladder may be associated with the pathogenesis of IC/PBS. However, the causative relationship has not been well investigated, and more solid evidence is needed.

EBV Infection Involves Chronic Bladder Inflammation and Nerve Hyperplasia in IC/BPS Bladders

In our institute, we had much experience in treating HIC with electrocauterization and partial cystectomy with augmentation enterocystoplasty [35, 36]. Hence, our pathologists could have experience in approaching bladder specimens from patients with HIC, especially large bladder specimens from partial cystectomy. In 2018, our pathologist Dr. Yung-hsiang Hsu had an insight into the histopathological characteristics of IC/BPS bladders that were similar to EBV-infected mucosa tissue. Similarly, a histopathology study investigated HIC bladders and found lymphoid aggregates/follicles [8•]. In the study, light chain-restricted B cells were detected in 9 of 46 HIC bladder biopsy specimens. Hence, the possibility of an early, minute, mucosa-associated lymphoid tissue (MALT) lymphoma in these HIC biopsy specimens could not be excluded. Previous studies have revealed the presence of EBV in 2.2% of specimens of gastric MALT lymphomas [37]. Furthermore, a recent study has shown single pathogenic mutations in EBV-positive MALT lymphomas compared with EBV-negative MALT lymphomas, including mutations in *IRF8*, *BRAF*, *TNFAIP3*, and *SMARCA4* [38]. This finding suggests that EBV infection may play a role in these lymphomas.

Previous studies have reported the presence of EBV in specimens of bladder cancer and upper urinary tract urothelial carcinomas [39, 40]. However, our previous study revealed EBV-harboring lymphocytes in bladder specimens from patients with IC/BPS [16••]. The Epstein–Barr-encoded RNA (EBER) by in situ hybridization positive rate in bladder specimens from patients with IC/BPS with HIC and without HIC (NHIC) was 50% and 8.6%, respectively, whereas none was found in control specimens. EBV DNA by qPCR was detected in 68.8% of bladder specimens from HIC patients and 16.7% of bladder specimens from NHIC patients (medium viral load 1836 copies/mL, range 216–75,144 copies/mL). In this cohort, EBV bladder infection was present in 87.5% of bladder specimens from HIC patients and 17.4% of bladder specimens from NHIC patients (total IC/BPS patients 46.2%). Immunohistochemical staining of CD3 and CD20 revealed that EBER-positive cells do not involve the bladder germinal center. Our first study suggested that EBV infection may be linked to the pathogenesis of persistent inflammation in IC/BPS bladders.

Furthermore, we investigated EBV infection type in IC/BPS bladders and how EBV infection involves the pathogenesis of IC/BPS. We examined 13 EBER-positive bladder specimens. Among them, 10 were positive for EBV nuclear antigen 1 (EBV latency infection marker), 6 were

positive for latent membrane protein 1 (LMP1, EBV latency infection marker), and 4 were positive for immediate-early protein BRLF1 (EBV lytic infection marker) [17••]. EBER/CD20 and EBER/CD3 double staining were performed on 3 HIC bladder specimens. All the 3 HIC bladder specimens were positive for EBER/CD20, but one HIC bladder specimen was positive for EBER/CD3. The flow cytometry study revealed that 5–18% of the total cell population in the HIC bladder specimens produced LMP1/CD19 or BZLF1/CD19 dual signals (EBV-infected B cells), and only 0–2.66% were positive for LMP1/CD3 or BZLF1/CD3 (EBV-infected T cells), indicating the coexistence of EBV persistence and reactivation in IC/BPS bladders and EBV infection in B cells. Furthermore, we performed RNA-seq-mediated transcriptome analysis for HIC bladders, and the results revealed enriched EBV infection-associated pathways in both KEGG pathways and gene set enrichment analysis.

In our first study, in situ hybridization revealed that EBER-positive cells were not presented at the germinal center of the HIC bladders [16••]. Since the germinal centers are generally considered to be the site of B cell aggravation [41], we supposed that the EBV infection in the HIC bladders was not involved with B cells, and the T cells might be the host of EBV infection. In fact, EBV infection in the germinal center is rare and were only identified in the immunocompromised patients [42]. Our following study with flow cytometry provided solid evidence to show that most EBV-infected cells were CD19 positive, and the B cells should be the main host of EBV infection in the HIC bladders [17••]. EBV-infected T cells in HIC bladders also had been identified with both double immunochemical staining and flow cytometry, but it was only a small part of EBV infection [17••]. The B cells are the primary targets of EBV infection in human, and most patients with EBV infection is asymptomatic. EBV infection in the T cells is unusual and suggests viral replication and the potential of malignant transformation [43]. In the patients with HIC, EBV infection mostly in B cells and few in T cells suggests that virus infection is still active and resulted in bladder persistent inflammation.

Besides inflammation, nerve hyperplasia and neurotrophin and sensory receptor upregulation have frequently been noted in IC/BPS bladders [44, 45]. However, few studies have investigated the origin of nerve hyperplasia and neurotrophins in IC/BPS bladders. Animal studies have reported that brain-derived neurotrophic factor (BDNF) overexpression induced neuronal changes that cause bladder overactivity and activate astrocytes to release TNF-α, thus aggravating neuroinflammation in the bladder [46, 47]. In humans, an association was observed between increased BDNF expression in the colonic mucosa and nerve hyperplasia and visceral hyperalgesia in irritable bowel syndrome [48]. Our experiment [17••] revealed BDNF overexpression in HIC bladders, and the flow cytometry results indicated that the BDNF originated from the B cells in the bladders. The in situ hybridization and immunohistochemistry double staining results revealed the colocalization of EBER/BDNF in the HIC bladder specimens, suggesting that EBV infection in B cells might induce BDNF overexpression in HIC bladders. Our further in vitro experiments proved that EBV infection in B cells resulted in significant BDNF expression, and the EBV nuclear proteins, especially EBNA-1, could work with human epigenetic modification enhancers to act on the BDNF promoter to initiate BDNF synthesis. Additionally, an animal study of the implantation of EBV-infected B cells into the bladder revealed nerve hyperplasia. Our results showed an association between EBV infection-induced BDNF overexpression and chronic bladder inflammation and nerve hyperplasia in HIC bladders.

Despite these findings, the mechanism by which EBV infects the human bladder is still unclear. Although EBV could infect human epithelial cells [49], it is unlikely that EBV that could directly cross barriers of the urinary tract and invade human bladders. We hypothesize that patients with EBV-associated IC/BPS may have previously had bacterial cystitis and bladder inflammation. Furthermore, some EBV-harboring lymphocytes get into bladders with hematogenous spread. With somehow unclear mechanisms, the EBV-harboring lymphocytes aggregate into the bladder lamina propria and finally cause persistent bladder inflammation.

Further Perspectives

Currently, most studies that investigated the presence of urinary tract viruses in patients with IC/BPS only enrolled a small number of patients and targeted several virus species. Thus, further studies including a large number of patients and screening the viral spectrum in the urinary tract, including bladder and urine, are needed. Viral culture, PCR, and high-throughput sequencing technology have respective advantages and should be used to validate the presence of urinary tract viruses in further studies. Furthermore, the association between viral infection and bladder histological/molecular changes should be investigated. Since EBV infection was presented in bladder specimens from patients with IC/BPS, using an antiviral treatment may have clinical benefits for the patients.

Conclusions

The presence of urinary tract viruses in patients with IC/BPS has been sporadically investigated. Early studies used viral culture to investigate urine and bladder tissue

samples from patients with IC/BPS, but viruses were rarely detected. Several studies used PCR and reported increased papillomavirus BKV and JCV viral load in urine samples from patients with IC/BPS. Recent studies of RNA sequencing revealed several enriched viral infection-associated pathways, suggesting that viral infection may involve the mechanism of IC/BPS. Our recent studies reported the presence of EBV in IC/BPS bladders, and EBV latency infection in B cells was associated with BDNF overexpression and nerve hyperplasia in bladders. Virus infection might be a pathogenic factor in some patients with IC/BPS, but the mechanism needs to be further investigated.

Author Contributions J.F.J. collected the data and wrote the manuscript. H.C.K supervised, provided critical suggestions and revised the manuscript.

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Declarations

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Competing Interests The authors declare no competing interests.

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