Interstitial Cystitis: Pathogenesis, Urinary Markers, and Experimental Models

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Abstract

Interstitial Cystitis (IC) is a chronic and painful inflammation of the bladder wall that most often causes frequency and urgency to urinate. Interstitial cystitis (IC) affects at least 20,000 to 90,000 women in the United States (1) and one tenth as many men. Patients with IC usually present with pelvic pain, urgency and frequency of urination, and tend to follow a waxing and waning course. The diagnosis of IC remains one of exclusion, based entirely on clinical and cystoscopic criteria. The cause of IC is unknown and to date, there is no effective treatment to cure this disease; however, the treatments that are available may help relieve the symptoms, which include oral and intravesical therapies and surgery as a last resort. The lack of effective research models has hindered the development and understanding of this debilitating disease. Herein we review the pathogenisis, markers, and possible in vitro models of interstitial cystitis.

INTRODUCTION

The NIH-NIDDK established a set of inclusion and exclusion criteria, originally meant to be for research protocols (2). These have evolved into the defacto criteria for clinical diagnosis (3). These guidelines include a history of pain or urgency, as well as cystoscopic evidence of petechial hemorrhages (glomerulations) or ulcers that extend into the lamina propria (Hunner's ulcers). Several etiologies of IC have been proposed, including changes in neuronal function, autoimmune reactivity, mast cell activation, defects in urothelium and infection. Although no one specific etiology for IC has yet been identified, the use of in vitro models has helped to demonstrate specific pathologies that may contribute to symptoms. The syndrome may have multiple etiologies, and may involve a number of pathologic processes, all of which result in a similar clinical picture. Thus, the most realistic models will likely be those that incorporate data from a variety of sources to form a multifactorial model of the disease. This article reviews the current theories of pathogenesis, and describes in vitro models used to study IC, and how they have been used to refine etiologic hypotheses.

METHODS

A periodical search was performed using PubMed to identify references pertaining to etiologies of IC, with a focus on the use of in vitro models. Search terms included cystitis, in vitro, model, interstitial cystitis and etiology. Articles published from 1980 to August of 2003 were included, with an emphasis placed on the more recent literature. The leading etiological hypotheses were identified as urine toxicity, occult infection, defective urothelium, neurogenic inflammation, mast cell activation and autoimmunity. These theories of pathogenesis are detailed below.

THEORIES OF PATHOGENESIS

TOXIC SUBSTANCES IN THE URINE

One of the leading theories of pathogenesis is that the urine of IC patients is itself carrying a toxic substance accounting for the disorder. This may be an abnormal substance, uniquely present in the urine of IC patients, or a normal constituent present at an abnormal concentration. This hypothesis is usually tested by exposing normal adult urothelial cells to samples of urine from IC and control patients. The proliferation of the cells is then assessed by measuring the uptake of ^{3}H-thymidine.

Studies of this type have yielded conflicting results. In one report, inhibition of colony formation by urine from healthy volunteers or women with IC was not significantly different (4). However, a limitation of this study was that fibroblasts were used rather than urothelium. Other studies have found no difference in urine toxicity, (5) or lymphocytic response (6) between interstitial cystitis patients and healthy controls. This study was unique in that it measured the release of

intracellular bound ^{51}Cr to assess cell death. The use of cell death as an outcome makes it possible to miss a sub lethal effect of IC urine on normal epithelial cells. For this reason, the effect on cell proliferation is more commonly used as an outcome.

Other studies have shown the presence of antiproliferative factor (APF), in the urine of IC patients that inhibits the proliferation of normal human bladder epithelial cells in vitro (7,8). The proliferation rate of explanted bladder cells from IC patients has also been shown to be significantly less than that of control subjects, suggesting an intrinsic abnormality in IC cell proliferation (9). APF has been shown to inhibit epithelial cell production of heparin-binding epidermal growth factor-like growth factor (HB-EGF) (10), a protein generated by, and stimulatory for, bladder epithelial cells in vitro (11). Not only is urine HB-EGF concentration decreased, but serum concentration is decreased as well (10). This could indicate that IC is a urinary tract manifestation of a systemic disorder. A recent study has shown consistent elevations of APF and EGF, and reductions of HB-EGF in IC patients of three different racial groups, suggesting that there may be some utility in using these proteins as biomarkers for IC patients (12).

The theory that IC urine contains toxic factors is also supported by the fact that bladder tissues treated with urine from IC patients showed increased expression of a stress protein (heat shock protein, 72 kDa HSP). Increases in this stress protein have also been observed after exposure to known harmful environmental stimuli. The precise role of stress proteins is unknown, but there appears to be a direct relationship between the induction of HSP and thermotolerance, suggesting a cytoprotective function (13).

In another study, fractions of normal urine were examined for toxic effects on bladder tissue in vitro. In the same system, the effects of Tamm-Horsfall protein (THP) were measured. THP, the most abundant protein in normal urine, is thought to be a cytoprotective factor that acts as a scavenger of cationic substances that have potential to injure bladder mucosa. Normal human urine contains cationic, low molecular weight components that, when separated from the bulk of urinary wastes, are cytotoxic to urothelial cells. This cytotoxicity can be blocked by pre-incubation with THP (14). This raises the question of whether the pathological process is a result of a deficiency cytoprotective factors rather than an excess of toxins.

DEFECTIVE BARRIER/UROTHELIUM

Another cytoprotective factor in the bladder is the glycocalyx. This layer of glycoproteins and glycosaminoglycans (15) covers the mucosal surface of the urinary bladder, and functions as a barrier to pathogenic microorganisms and toxins in the urine. A defect in this protective layer could permit pathogens leak into the subepithelial space and the muscularis, possibly injuring muscle and/or initiating sensory stimulation causing symptoms of IC. In vitro analyses of bladder biopsies from IC patients have shown abnormalities of the glycocalyx, most notably the deficiency or absence of a particular glycoprotein component (GP1). GP1 was significantly decreased in 61% and absent in 35% of IC patients when compared with control patients (16).

OCCULT INFECTION

Although the symptoms of interstitial cystitis are similar to those of bacterial cystitis, urinalysis and urine cultures routinely demonstrate no evidence of infection. Even more sensitive examinations (nested PCR assays for bacterial DNA) of bladder tissue from IC patients and healthy women have failed to demonstrate a specific bacterial or viral cause (17, 18, 19, 20, 21). It has been proposed that IC may develop as a consequence of urothelial injury that persists after infectious agents are no longer present. The proliferative ability of progenitors of urothelium cells is increased in IC. A possible mechanism may be that recurrent injury, caused by infection and inflammation, selects for these highly proliferative stem cells, triggering chronic changes in the architecture of the urothelium. These chronic changes may then explain symptoms that persist well after an infection has resolved (22).

NEUROGENIC INFLAMMATION

Histopathologic changes evident in IC include submucosal inflammation along with thinning or ulceration of bladder epithelium (23). Certain inflammatory mediators, such as substance P and bradykinin also function as nociceptive neurotransmitters. IC patients have been shown to exhibit a hyperinnervation of the bladder (24), as well as an increased number of substance P containing nerves (25). This combination may help to explain the chronic bladder pain suffered by IC patients. Bradykinin, substance P and histamine are capable of causing contractions of urinary bladder smooth muscle, as well as enhancing neurogenic contraction of the urinary bladder by affecting the purinergic component of bladder efferent fibers (26). In light of the fact that a purinergic component of neurotransmission is much

more prominent in women with IC (27), it would make sense that an increase in levels of these mediators might produce symptoms of urgency and frequency.

MAST CELL ACTIVATION

Substance P and Bradykinin are also powerful activators of mast cells. Increased numbers of mast cells have been demonstrated within the detrusor layer of the bladder in IC patients (28, 29). They have been found in close apposition to neurons (30), suggesting that they may play a role in the pathogenesis of IC. Mast cell secretion is stimulated by estrogen (31, 32), and inhibited by tamoxifen (32), which may explain the increased incidence of IC in females. It has also been shown that mast cells from the bladders of IC patients are far more responsive than those isolated from normal tissue (33). These findings suggest that the bladder environment in IC may induce a phenotypic change in the mast cell, thus influencing its rate of maturation and proliferation.

AUTOIMMUNITY

Many of the clinical features of IC suggest that autoimmunity may play a role in the chronic inflammatory component of the disease. The symptoms are chronic, with exacerbations and remissions. Autoantibodies have been detected in the sera of IC patients to a greater extent than in controls (34, 35), and IgA antibodies have been detected on the surface of bladder epithelium in IC patients (36). The distinction between an autoimmune phenomenon and generalized chronic inflammation lies in the specificity of the immune response. In vitro studies evaluating autoimmune components have failed to show any convincing evidence of any primary immunologic disorder (37, 6).

EXPERIMENTAL MODELS

The development of new and more effective treatments modalities for IC has been severely hampered by the lack of experimental models. Several in vivo models of IC that have been reported in the literature include a mouse model (38), Guinea pig model (39) and a naturally occurring feline model (40). While these models provided a valuable foundation for the investigation into experimental models of IC, they have not become widely used and sighted in the medical literature. With this reason in mind we have begun investigation into developing an in vitro model of IC . We used the UROtsa cell line which has been used as a normal control for bladder tissue in vitro. The UROtsa cell line was isolated from normal urothelium lining the ureter of a 12 year-old girl and was immortalized by using a temperature-sensitive SV40 large T-antigen gene construct (41). The UROtsa cell line was suspended to a cell concentration of 1 x 10^5 cells/ml in varying media pH conditions (pH = 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0). Cells were added to the assigned wells in a 96 well microtiter plate in a volume of 100ul (1 x 10^4 cells/well). The microtiter plates were be incubated at 37 Deg. C, 5% CO_2 for 24, 48, and 72 hours respectively. After 24, 48 and 72 hours, the cells were assayed for cell viability using the MTT colorimetric assay for cell viability (42). Preliminary results have shown that significant anti-proliferative effects were observed among the varying pH conditions tested in the UROTsa cell line (P<0.001, ANOVA, Table 1).

Figure 1

Table 1.

Table 1.

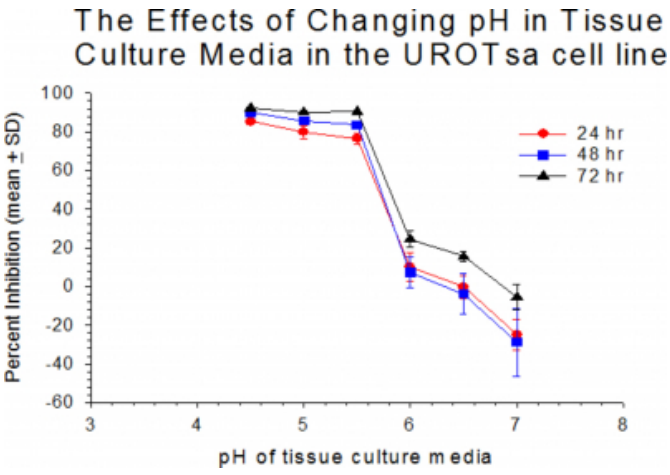
	Percent Cell Growth Inhibition		
	24 Hours	48 Hours	72 Hours
pH	Mean(S.D.)	Mean (S.D.)	Mean (S.D.)
7.0	-25.1(8.1)	-28.6 (17.6)	-5.5 (6.7)
6.5	-0.3(5.9)	-3.8 (10.5)	15.5 (2.3)
6.0	10.1 (7.4)	7.3 (8.1)	24.5 (4.1)
5.5	76.5 (2.7)	83.7 (1.9)	90.5 (0.7)
5.0	79.8 (3.2)	85.6 (0.9)	90.2 (0.9)
4.5	85.3 (1.3)	89.8 (0.8)	92.2 (0.6)

ANOVA = p<0.001 at 24, 48, and 72 hours

The reduction in cell proliferation was pH dependent, however it was not time dependent. No increase in response was observed among the 3 time-points tested (24, 48, and 72 hours, Figure 1).

Figure 2

FIGURE 1:



The statistically significant anti-proliferative effects of pH exhibited here suggest that urine pH could be a contributing

cause of or product of Interstitial Cystitis. Further research is needed, but these results suggest that reduction of media pH in vitro could provide a model of IC that could prove to be invaluable for the investigation of new and improved treatment modalities

CONCLUSIONS

Potential areas of further research involve mapping out the complex interaction of inflammatory mediators in the IC microenvironment. Urothelial cells in IC express different surface proteins than do healthy cells exposed to cytokines (43). This may suggest that a specific type of inflammation is present in interstitial cystitis, or an inappropriate response of the urothelium. The in vitro studies have also suggested a number of markers (APF, HP-EGF, EGF and mast cell phenotype) that may have diagnostic value. An analysis of the specificity and sensitivity of these markers may prove invaluable, considering the fact that IC is currently a diagnosis of exclusion. An assessment of the effects of serum-based medium would help to allow researchers to control for any related confounding factors, thus improving the accuracy of in vitro models. Lastly, samples from patients at different stages of the disease may help us to gain a longitudinal view of the pathologic process, although the waxing and waning nature of IC may complicate efforts.

The etiology of IC remains elusive. The use of in vitro models examine various elements of the disease has been quite useful, although they do have some inherent limitations. In particular, they cannot address certain issues involving anatomic location or patient sensitivity. Another limitation is that most in vitro models require the use of serum-supplemented culture media, which may influence the data. The use of growth inhibition and cytotoxicity, may not be appropriate endpoints for assessing the disease. Techniques of re-creating disease conditions are becoming more and more realistic however, and with each new study, our etiological picture becomes more clear. What are now separate theories will likely begin to blend together into one model explaining the symptomatology, histopathological findings and unique characteristics of IC described in the literature.

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