The Mast Cell in Interstitial Cystitis: Role in Pathophysiology and Pathogenesis

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Current evidence from clinical and laboratory studies confirms that mast cells play a central role in the pathogenesis and pathophysiology of interstitial cystitis (IC). In this article, we focus on the role of the mast cell in IC and examine the ways in which mast cells and other pathophysiologic mechanisms are interrelated in this disease. Identifying the patients with IC who have mast cell proliferation and activation will enable us to address this aspect of disease pathophysiology in these individuals with targeted pharmacotherapy to inhibit mast cell activation and mediator release. UROLOGY 69 (Suppl 4A): 34–40, 2007. © 2007 Elsevier Inc.

Interstitial cystitis (IC), a condition characterized by symptoms of urinary urgency/frequency, suprapubic and pelvic pain, and dyspareunia, has a profound negative impact on patients' quality of life.[1] The pain associated with IC is not uncommonly the most bothersome of the symptoms; this has recently led to the suggestion that IC should be classified as painful bladder syndrome (PBS).[2] Significant clinical overlap exists between IC and several other disease states, including overactive bladder syndrome (OAB), especially "OAB-dry"; chronic cystitis/urethral syndrome; chronic pelvic pain (CPP) in women; and CPP syndrome (CPPS)/chronic prostatitis (CP) in men.[3–5]

The pathogenesis and pathophysiology of IC have been widely studied in humans and in various animal models (spontaneous as well as experimental).[6–8] The impetus for this research was largely the outcome of a 1987 landmark symposium on IC organized by the Interstitial Cystitis Association (ICA) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and the publication that same year of the first journal supplement on IC in *Urology*.

IC is a heterogeneous syndrome and current pathophysiologic concepts center on the role of the urothelial cell (increased permeability, neurotransmitter/neuropeptide release, etc.), neurogenic inflammation with bladder sensory nerve upregulation, and mast cell involvement in neuroimmunoendocrine inflammation. This review focuses on the role of the mast cell in IC/PBS and examines the interrelation between mast cells and other pathophysiologic mechanisms described elsewhere in this supplement.

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OVERVIEW OF MAST CELLS

Mast cells are multifunctional immune cells that develop from a specific bone marrow progenitor, migrate into tissue perivascular spaces, and acquire various characteristics as a result of microenvironmental conditions.[9,10] Mast cells are involved in allergic and late-phase reactions, in which they are stimulated by crosslinking of immunoglobulin (Ig) E bound to surface receptors (FcϵRI) and by specific antigens.[10] Mast cells are also involved in innate immunity and autoimmunity[10,11] and in neuroinflammatory disorders such as asthma, rheumatoid arthritis, and IC.[12] Mast cell activation can be triggered by nonimmunologic stimuli such as bacteria, chemicals, kinins, neuropeptides such as substance P (SP), and acetylcholine (ACh).[6,10]

Mast cell mediators are granule-stored, presynthesized molecules (eg, heparin, histamine, proteases, phospholipases, chemotactic substances, and cytokines) or are synthesized de novo (eg, cytokines, especially interleukin-6 [IL-6]; leukotrienes; prostaglandins; nitric oxide [NO]; and tumor necrosis factor–α [TNF-α]).[9,13] Damaged or dysfunctional urothelial cells produce cytokines, such as stem cell factor (SCF), that can stimulate proliferation and/or activation of mast cells.[6] Mast cells in IC are maximally activated by SCF.[14,15] Nerve growth factor (NGF), which is increased in patients with IC,[16] is a known mast cell stimulus. Anaphylatoxins, antibody light chains, aggregated IgG, bacterial or viral superantigens, and adherent *Escherichia coli* can activate mast cells, possibly through toll-like receptor–2 (TLR-2), stimulated by lipopolysaccharide (LPS).[6,17]

Vasoactive and inflammatory mediators secreted by mast cells may explain many IC symptoms.[6,12] Tryptase, which is increased in the urine of patients with IC, causes microvas-

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cular leakage and stimulation of protease-activated receptors (PARs), with resulting inflammation and submucosal neuronal hyperexcitability.[18,19] Vascular endothelial growth factor (VEGF), another mast cell mediator, is vasodilatory, and its overexpression in IC bladders may contribute to the hypervascularity and glomerulations characteristic of IC.[20]

The 2 major mast cell subtypes, connective tissue mast cell (CTMC) and mucosal mast cell (MMC), differ in histochemical properties, type of granule-associated proteoglycans, neutral proteases and cytokines, morphology, sensitivity to secretagogues, and susceptibility to inhibitory drugs.[9,15] Mucosal mast cells are found most often in the bladder and the gastrointestinal tract.[5] They are susceptible to aldehyde fixation but do not stain with acidified toluidine blue or Alcian blue counterstained with safranin O.[21] Mucosal mast cells are, therefore, not easily identified in bladder tissue fixed in 10% formalin.[6,21]

Vasoactive, nociceptive, and proinflammatory molecules released from activated mast cells produce neuronal sensitization and secretion of neurotransmitters that further stimulate mast cells.[6] The urothelium is no longer regarded as a passive barrier but as an active component of the bladder wall that exhibits specialized sensory and signaling properties.[22] This vicious circle contributes to the chronic and painful symptoms associated with IC/PBS. It has been suggested that IC is a visceral neuropathic pain syndrome mediated by nerve upregulation in the pelvis, spinal cord, and brain.[1,6]

NEUROIMMUNE INTERACTIONS OF MAST CELLS

Neuroimmune interaction involving mast cells in the bladder may explain the sensory neuronal hyperreactivity and neuropathic pain seen in IC/PBS.[23,24] Bladder inflammation due to viral pseudorabies in the central nervous system (CNS) does not develop in mast cell–depleted mice, which suggests neuronal mast cell interaction or communication.[25] Increased SP has been reported in bladder biopsy specimens of patients with IC, and SP participates in neuronal mast cell communication.[26,27] Mast cells have a close spatial relation with nerves in normal bladders and in bladders with IC.[28,29] Intravesical administration of SP induces bladder mast cell inflammation via neurokinin (NK)-1 receptors.[30] In animal models of intravesical administration of ovalbumin in sensitized rats and mice with bladder inflammation induced by LPS and SP, mast cells have been shown to participate in bladder inflammation and inflammatory mediator release.[31,32] Nuclear transcription factor–κB, the key regulator of inflammatory gene expression, is activated in IC and in response to TNF-α released from activated mast cells.[13,33] SP, neurotensin, NGF, and SCF all activate mast cells.[19,34]

Stress is known to increase symptoms in patients with IC,[35] and family genetic linkage studies have shown that panic disorder is associated with IC, possibly through neurogenic inflammation involving mast cells.[36,37] In restraint and cold-stress animal models, bladder mast cells are increased and activated.[38,39]

Corticotropin-releasing hormone (CRH), secreted from the hypothalamus under stress, regulates the hypothalamic-pituitary-adrenal axis and is expressed peripherally in the spinal cord, dorsal root ganglia, sympathetic ganglia, and mast cells.[40] CRH secreted from these non-CNS sites has proinflammatory actions that may be mediated via mast cell activation.[41] In humans, CRH administration causes mast cell–mediated peripheral vasodilation and flushing, and this may contribute to the skin hypersensitivity seen in patients with IC.[40–42]

HUMAN STUDIES OF MAST CELLS IN INTERSTITIAL CYSTITIS

Increased Numbers (Mastocytosis)

Evidence from human studies of mast cell involvement in IC is summarized in Table 1. As summarized by Theoharides *et al.*,[6] most studies in humans have shown an increase in mast cell numbers and activation in patients with IC. Varying reports of mast cell numbers and activation in the submucosa versus detrusor layers of the bladder and in "ulcer" versus "nonulcer" IC are due to (1) differences in mast cell stains used (eg, Giemsa and toluidine blue identify only nonactivated and nondegranulated mast cells), and (2) methods of tissue fixation (eg, formaldehyde fixation fails to fully recognize MMCs stained with toluidine blue). Mast cells are more consistently increased in classic IC with Hunner ulcers.[6,15] In nonulcer IC, reports on bladder mast cells show large standard deviations, which raises the possibility of methodologic problems or the possible existence of heterogenous patient subgroups.[6] Transitional cell bladder carcinoma is associated with increased numbers of mast cells, and the use of patients with bladder cancer as controls adds a confounding variable when results of these studies are interpreted.[6,15]

An early report described increased numbers of partially or completely degranulated mast cells in the detrusor but not in the submucosa of patients with IC.[43] However, a subsequent study found significant ($P < 0.01$) increases in the number of mast cells in the detrusor and submucosa layers of patients with IC when compared with controls; the increase was larger in the detrusor layer.[44] It has been suggested that mast cell counts >20 cells/mm^2 in bladder muscle have an 88% diagnostic specificity and a 95% diagnostic sensitivity for IC.[45]

Tissue fixation techniques that allow identification of MMCs and CTMCs reveal an increased number of mast cells in the detrusor and submucosa layers in IC.[46] When compared with the control group, significant mastocytosis was noted in the detrusor layer but not in the submucosa layer in IC, with higher levels of degranulation noted in the detrusor layer (56.4% vs 44.6%) ($P < 0.05$).[47]

Johansson and Fall[48] reported mast cell increases in both layers of the bladder wall in patients with ulcer IC.

Table 1. Evidence from human studies of mast cell involvement in interstitial cystitis (IC)

- Light and electron microscopy
  - —Increased number of mast cells (submucosa and detrusor)
  - —Activation and degranulation
- Tryptase immunohistochemistry
  - —Increased number of mast cells
- Urinary mast cell mediator increase
  - —Methylhistamine
  - —Tryptase
  - —Other mediators
- Mast cell sensory nerve communication
  - —Spatial proximity
  - —Increased SP immunoreactivity
  - —Increased NK-1 receptor expression
- Mast cell activation in IC and overlapping conditions
  - —Irritable bowel syndrome
  - —Endometriosis
  - —Chronic prostatitis

NK-1 = neurokinin-1; SP = substance P.

In another study that compared patients with IC or bacterial cystitis with controls, 146 mast cells per 10 higher power fields were identified in the urothelium/submucosa of patients with IC, 97 in patients with bacterial cystitis, and 51 in controls.[28] Corresponding levels in the detrusor were 170, 45, and 46 mast cells, respectively.

Tryptase is a unique protease that is present in both types of human mast cells. Using tryptase immunocytochemistry, the most accurate technique for identifying human mast cells, a recent study[49] confirmed previous findings of mast cell increase in patients with nonulcer IC.[50] A European study using similar immunocytochemical techniques showed a 6- to 10-fold increase in mast cells in classic/ulcer IC and a 2-fold increase in patients with nonulcer IC compared with controls.[15] It is well recognized that IC has significant overlap with CPP in women, and that endometriosis has been considered a common cause of this condition.[1,4] Recently, this has been challenged by new data that show the bladder as the source of CPP[4] (see also the article by Stanford *et al.*[51] in this supplement). Increased numbers of activated mast cells have been found in pelvic endometrial deposits from women with CPP.[52]

Mast cells are increased in a number of gastrointestinal diseases (eg, irritable bowel syndrome) that occur with a higher prevalence in patients with IC than in controls.[53] A common pathophysiologic mechanism for these diseases may be mast cell activation with sensory nerve upregulation.[54,55]

Activation

Light microscopy techniques do not fully reveal highly activated or degranulated mast cells.[6,15] This may explain the erroneously low mast cell counts reported in some early studies. Activated mast cells are best identified ultrastructurally and by assessment of their mediator/secretagogue release.[18,50,56] To avoid the problems and confounding variables associated with light microscopy identification of mast cells, the Tufts University group conducted a careful electron microscopic study (the only study of its kind to date) of bladder biopsy specimens from patients with IC; this convincingly revealed increased numbers and activation of mast cells in patients with IC compared with controls.[50,56] Mast cell activation with release of cytoplasmic granules occurred in 30% of controls, 24% of patients with transitional cell carcinoma, and 80% of patients with cystoscopically confirmed IC, as defined by NIDDK criteria.[50] Ultrastructural evidence of intragranular activation is distinct from the typical massive degranulation associated with allergic or anaphylactic reactions and is thought to be due to "differential" release of secretory mediators.[57]

When challenged immunologically in vitro, mast cells from IC were more responsive than normal cells in terms of histamine release.[6] SP and compound 48/80 are weak triggers for bladder mast cells, whereas the ACh analogue carbachol is a potent stimulus. Bladder mast cells from patients with IC are more responsive to secretagogues such as IgE, antigen, and ACh.[6,58]

Many premenopausal women with IC have perimenstrual worsening of their irritative voiding and pelvic pain symptoms.[1] Bladder mast cells from women with IC exhibit increased expression of high-affinity estrogen receptors, and estrogens induce proliferation of bladder mast cells in a guinea pig model of IC.[6,59]

Urine Mast Cell Mediators

The most compelling support for mast cell involvement without light microscopic evidence of mastocytosis or degranulation is the ability of mast cells to secrete mediators, especially biogenic amines and cytokines.[6] Release of IL-6 can be induced from mast cells by bacterial LPS without concomitant histamine release. This finding may be particularly relevant to IC, in that IL-6 is elevated in the urine of patients with IC and IL-6–positive

cells are present in the mucosal and detrusor layers of their bladders.[15,60] High urine IL-6 levels are associated with severe inflammation in patients who respond to bladder hydrodistention,[28] and IL-6 is also expressed in the urothelium of bladder metaplastic lesions and classic IC.[15]

In patients with IC, histamine levels are increased in the bladder wall,[45] and urine histamine is elevated after bladder hydrodistention.[61] The major histamine metabolite 1,4-methylimidazole acetic acid is increased in the urine of patients with IC with detrusor mastocytosis, and methylhistamine is significantly elevated in the urine (24-hour urine collection) of patients with nonulcer IC.[62,63] Tryptase, the specific human mast cell proteinase enzyme, is also elevated in the urine of patients with IC.[18]

MAST CELLS IN ANIMAL MODELS OF INTERSTITIAL CYSTITIS

A number of in vivo animal models, induced and spontaneously occurring, have been used to elucidate the pathogenesis of IC. These have been admirably summarized and can be broadly grouped into models that use noxious intravesical and systemic chemical stimuli, noxious environmental stimuli, and immune sensitization, and the naturally occurring model of feline IC.[8] Most induced models largely reflect acute noxious injury to the bladder and provide insights into nonspecific bladder responses to injury, whereas immune and feline IC models more accurately reflect the chronicity and time course of IC in humans.[6,8]

Mast cell proliferation is a primary feature in an autoimmune mouse model of IC. When Balb/cAn mice are immunized with syngeneic bladder homogenate, bladder edema, fibrosis, and mast cell accumulation develop.[64] Type 1 fimbriated bacteria induce mast cell degranulation and histamine release in mice,[17] a finding that is relevant to the pathophysiology of IC. Dormant microbial DNA that suggests cell wall–deficient bacteria has been described in IC, and many women with IC have antecedent diagnoses of urinary tract infection and chronic cystitis.[1,65] The recent demonstration of bacteria within the urothelial cell cytoplasm in animals after bacterial cystitis suggests a link between the bacteria, the urothelium, and mast cells.[66]

In feline IC, a condition in which the cat bladder shows signs similar to those in humans with IC, bladder mast cells are increased.[8] In a guinea pig bladder animal model, antigen challenge after in vivo sensitization with ovalbumin resulted in histamine release and mast cell degranulation.[67] Isolated guinea pig urinary bladder also responded to SP, NK-A, vasoactive intestinal peptide, and bradykinin with histamine release. Intravesical ovalbumin administration in sensitized rats induced bladder plasma extravasation, which was blocked by prechallenge degranulation of mast cells.[31] In another model, cystitis induced by intravesical administration of SP or LPS caused bladder plasma extravasation that could not be reproduced in mast cell–deficient mice.[32] Moreover, rat neurogenic cystitis induced by invasion of the CNS by pseudorabies virus depends on bladder mast cell activation.[25]

SP and the neurotransmitter ACh activate rat bladder mast cells in vitro.[68] In the rat urinary bladder, SP-positive nerves are deleted by bladder distention, a therapy for IC. In patients with hypersensitive bladder disorders, intravesical capsaicin, which acutely stimulates neuronal release of SP, relieves pain.[6] Bladder and intestinal mast cell activation in rats results from acute psychological stress.[38,69] CRH-induced mast cell activation mediates these actions,[40] leading to proinflammatory effects. Urine release of histamine, rat mast cell protease–1, and IL-6, an action blocked by intravesical pretreatment with 0.4% sodium hyaluronate, also results from acute psychological stress.[6]

Mast cells have been shown to be essential for the inflammatory response and gene expression in experimental cystitis, and mast cell nerve communication is increased in cystitis.[70] NK-1 receptor expression has been demonstrated in patients with IC[71] and affects the number of mast cells in the bladder.[6,29] Animal models of IC that have shown mast cell involvement are summarized in Table 2.

CONCLUSION

Current evidence (human and experimental) from electron microscopy and modern immunohistochemical staining techniques confirms a central role for mast cells in the pathogenesis and pathophysiology of IC. Identification of patient subgroups with IC and mast cell proliferation and activation will allow targeted pharmacotherapy with drugs that inhibit mast cell activation and mediator release.[72,73] No clinically available compounds effectively block mast cell activation. Chondroitin sulfate[74] and the flavonoid quercitin[75] both have synergistic action in inhibiting mast cells and have been incorporated into the dietary supplement CystoProtek (Algonot Inc., Sarasota, FL). In a pilot open-label study, a 6-month trial of CystoProtek treatment was associated with significant symptom reduction in 37 patients with IC.[73,76]

Mast cells may be activated by a number of mechanisms within the bladder wall. Increased urothelial permeability with influx of potassium ions may lead to sensory afferent nerve upregulation with stimulation of mast cell activation, which, in turn, could further activate the afferent nerves in a vicious circle.[77]

Recent evidence from humans and from animal models has shown that the urothelium can release a number of neuropeptides (eg, purinergic, NGF) and neurotransmitters that may activate submucosal afferent nerves and mast cells.[22,77,78] These changes in urothelial permeability, urothelial activation, sensory nerve stimulation, and mast cell activation are complex and highly interrelated with multiple and simultaneous positive and negative

Table 2. Animal models of interstitial cystitis (IC) showing mast cell involvement

- Antigen-induced cystitis (mouse)
- Autoimmune cystitis (mouse)
- Experimental cystitis (mouse)
- Systemic SP/LPS (mouse)
- Acute cold-stress model (rat)
- Acute immobilization stress (rat)
- CNS-induced (pseudorabies) neurogenic cystitis (rat)
- Neurogenic inflammation (guinea pig)
- Ovalbumin-sensitized immune cystitis (guinea pig and rat)
- Feline IC (cat)

CNS = central nervous system; LPS = lipopolysaccharide; SP = substance P.

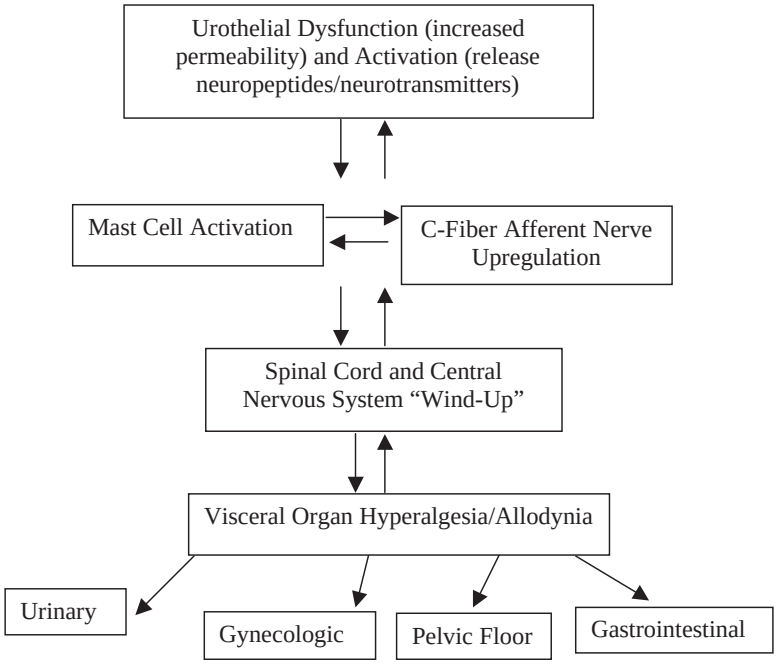

Figure 1. Pathogenesis of interstitial cystitis—an integrated pathophysiology.

feedback loops (Figure 1). This vicious circle contributes to the chronicity of voiding and pain symptoms of IC, as well as the complex neuroinflammatory and neuroimmunoendocrine changes in the bladder wall of patients with IC.

Once the sensory nerves in the bladder are upregulated, neurons in the dorsal post ganglia and spinal cord also release tachykinins (including SP), leading to a state of neurologic "wind-up" manifested by visceral allodynia and hyperalgesia in the bladder and adjacent pelvic organs (gastrointestinal, gynecologic). This explains why many patients with IC have pelvic floor dysfunction, gynecologic symptoms such as dyspareunia and vulvodynia, and gastrointestinal conditions such as irritable bowel syndrome.

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