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Speaker Abstracts

Interstitial Cystitis: Past and Future

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The diagnosis and management of interstitial cystitis (IC) has had a long and varied history. Leading theories for the pathogenesis of IC include changes in urothelial permeability, increased activity of mast cells, neural-immune mechanisms, plasticity in nervous system, and infectious etiologies. These postulated etiologies have led to a variety of treatment regimens, none of which uniformly eradicate the symptoms of urinary frequency, urgency, nocturia and/or pain. Treatments for interstitial cystitis have historically ranged from pharmacological therapies that relax or anesthetize the bladder to antihistamines or drugs that decrease urothelial permeability. Intravesical administration agents such as DMSO, clorpactin, and BCG have shown some encouraging results in select patients. Pain medications including tricyclic antidepressants have been used with varying success. The surgical history of interstitial cystitis has been dismal for the control and pain. Sacral rhizotomies, augmentation cystoplasties, and cystectomies have failed to relieve symptoms in the majority of patients. More recently, medical devices such as electromagnetic stimulation (Neotonus) or sacral nerve root stimulation (Interstim) have shown some promise. Unfortunately, few of these drugs or devices have been subjected to placebo-controlled randomized trials. Moreover, a consensus on outcome measures is lacking for clinical trials. Thus, the treatment of IC is often not practical relying on evidence based medicine.

Recent studies on the natural history of IC have shed insight into potential risk factors, associated conditions, and progression of disease. On the basic science front there appears to be a surprising convergence of data suggesting common neural mechanisms associated with chronic pain, urge/frequency symptoms, and nocturia. Regardless of the etiology (infection, inflammatory, neural) for IC, these similar mechanisms may be involved leading to the limited repertoire of behaviors that bladder can exhibit. Pain and discomfort in IC patients may not be identical based on differing etiologies. Two distinct pathways exist for the initiation and maintenance of chronic pain. One pathway leading to "neuropathic pain" involves sensitization of nociceptive pathways due to nerve growth factor (NGF) with activation of neurons in the CNS and silent C-fibers in the periphery. Afferents contributing to neuropathic pain exhibit changes in tetrodotoxin (TTX-5) sensitivity to the SCN-3A channel. Another pathway leading to chronic inflammatory pain is associated with activation of silent C-fibers. In contrast, molecular events in response to inflammation cause increased NGF and expression of tetrodotoxin (TTX-R) in resistant receptors as peripheral or central projections. Expression of these two distinct isoforms Na channels has been linked to spontaneous, burst firing of nerves, and a lowering of thresholds for activation. These molecular changes underlie allodynia (non-

painful stimuli causing pain) and hyperesthesia (heightened response to painful stimuli). By analogy, low volume filling of the bladder is painful and non-painful intravesical contents such as potassium cause discomfort. Changes in primary afferents then lead to plasticity in second order neurons with the expression of a protein kinase C isoform (PkC). Elimination of the painful focus may fail to deactivate these neurons in CNS leading to a behavioral correlate such as phantom pain. Simple blocking or severing a neural pathway is unsuccessful for chronic pain conditions because the abnormal focus of neural activity has been shifted into the central nervous system. Animal models for chronic cystitis are consistent with this model.

Treatments of neuropathic versus inflammatory conditions differ. Neuropathic pain fails to respond to opiates or prostaglandin inhibitors. Rather, anticonvulsive drugs such as neurontin are of modest benefit. In contrast, inflammatory pain responds to COX2 inhibitors as well as opiates. It is possible that one or both mechanisms may play a role in the generation of discomfort and even urgency and frequency with IC. Only by understanding the molecular changes associated with the response of bladder to a variety of conditions leading to overactivity can new therapies be developed. The explanation for why certain patients are predisposed to conditions such as IC, prostatodynia, fibromyalgia, and irritable bowel syndrome may be uncovered through genomics. In the absence of identifiable etiologic factors, it is likely that future symptomatic treatments for IC will involve novel approaches relying on turning off activated pain pathways and targeting specific neurotransmitter receptors or second messenger based on pharmacogenomics.

Increased Plasma Norepinephrine Concentrations in Cats With Interstitial Cystitis

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The symptoms of IC seem to be exacerbated by stress, suggesting the involvement of some aspect of the stress effector systems, which include the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. Although previous studies have found abnormal vasomotor tone, and increased bladder sympathetic neuron density and urine norepinephrine (NE) excretion in IC patients, no data concerning plasma catecholamine concentrations or HPA axis function in patients with IC have been reported.

To further evaluate the role of the sympathoneural and sympathoadrenal systems and HPA axis in cats with feline IC (FIC), we measured baseline plasma concentrations of catecholamines and their metabolites, and baseline and CRH-stimulated plasma ACTH and cortisol concentrations to assess the response of the HPA axis to infusion of corticotropin releasing factor (CRF).

Eight healthy cats and 8 cats with FIC were anesthetized and catheters were placed in the external jugular vein. 6 hours after recovery, samples were obtained for HPLC analysis of plasma concentrations of NE, dihydroxyphenylglocol (DHPG), epinephrine (E), dihydroxyphenylalanine (DOPA), dopamine (DA), and dihydroxyphenylacetic acid (DOPAC). In 4 cats in each group, 1m g ovine CRH per kg. body weight was infused, and blood samples collected at intervals for 120 minutes for determination of plasma adrenocorticotropic hormone (ACTH) and cortisol concentrations.

Significant increases in plasma NE and DHPG and a trend toward increased E were found, whereas no effect of FIC on DOPA, DA, DOPAC, ACTH or cortisol was identified.

These results support and extend previous studies identifying an increase in sympathetic activity in cats with FIC. Despite the alterations in sympathetic activity, no effect of FIC on HPA-axis function could be identified. In humans, it has been proposed that IC might be a disease within the spectrum of chronic fatigue and pain syndromes that appear to preferentially afflict women. These syndromes are characterized by decreased sympathetic tone and blunted sympathetic and HPA-axis responsiveness. The present results, previous studies of sympathetic function in IC patients, and our inability to find abnormalities of HPA-axis function suggest that the pathogenesis of IC may be different from other syndromes in the proposed spectrum.

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Interstitial Cystitis— A Chronic Visceral Pain Syndrome

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Interstitial cystitis (IC) shares many features with other chronic non-malignant visceral pain syndromes. In clinical practice much emphasishas been placed on finding a specific etiology and specific pathological markers for the disease and on identifying specific events that precipitated IC. This conceptualization has influenced clinical treatment approaches for IC and has not resulted in significant progress in this area so far. An additional approach is suggested, based on the conceptualization of three hypotheses: (1) a spectrum of different insults can lead to chronic visceral pain in patients suffering from IC, (2) different underlying pathogenic pain mechanisms may require different pain treatment strategies for patients diagnosed with IC, (3) multiple different pathogenic pain mechanisms may coexist in the same patient requiring several different pain treatment strategies (perhaps concomitantly) to successfully treat chronic visceral pain associated with IC. This concept is likely to lead to new insights into the pathophysiological mechanisms of IC and to novel treatment avenues for patients suffering from IC and — in a broader view — also for patients with other chronic visceral pain syndromes.

Stimulation of Human Urothelial Cell Proliferation by Estrogen Receptor Activation

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Urothelial cells have been shown to express both estrogen receptor (ER) subtypes, a and b. We examined whether the activation of ER stimulates cell proliferation and whether this effect is mediated by nerve growth factor (NGF). The experiments were performed using human urothelial cells immortalized by human papillomaviruses E6 (E6 cells) and cell proliferation was determined using an alamarBlue assay (TREK Diagnostic Systems). The E6 cells were seeded in 96 well plates, incubated with different agents and cell proliferation was determined every 2 hours. Results: 1) The selective ER-a agonist 16 alpha-iodo-17beta-estradiol (16 IE2) stimulated cell proliferation by 6.3 fold (10 nM) as compared to 4.3 fold increase in controls, 8 hr after the drug was applied. Similarly, the selective ER-b agonist genistein stimulated cell proliferation by 6.6 fold (100 nM). 2) NGF (100 ng/ml) also stimulated increased cell proliferation (6.5 fold). 3) Cell proliferation was greatly inhibited by specific NGF antiserum. 4) Stimulation of cell proliferation by either 16 IE2 or genistein appeared to be inhibited by specific NGF antiserum. 5) Western blotting and/or immunocytochemistry revealed expression of ER-a and -b, Trk A (high affinity NGF receptor), p75 (low affinity NGF receptor), and NGF in E6 cells. Taken together, these results indicate that activation of either ER-a or -b stimulates cell proliferation. NGF may exert an endocrine effect and function as a growth factor for E6 cells. Suppression of cell proliferation in response to ER stimulation by NGF antibody suggests that the effects of ER activation on cell growth may be at least partly mediated by NGF.

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Gene-Regulation During Bladder Neurogenic Inflammation

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The central hypothesis of this work is that, regardless of the cause (LPS, substance P [SP], or antigens), bladder inflammatory responses follow a common pathway which involves: activation of mast cells and sensory nerves, release of SP, and activation of substance P receptors.

Objective: We have characterized, at the morphological level, bladder inflammatory responses to three different stimuli (LPS, substance P, and antigen challenge). The major limitation of the original study is that morphological alterations are measured 24 hours following urinary bladder stimulation. This timepoint was chosen to represent the peak of cell migration during acute inflammatory response. However, most of the alterations observed may not represent a direct tissue response to the stimulus. Indeed, both resident and migrating cells often release an array of substances that modulate inflammation.

Methods: In addition to morphologic alterations, we used a mouse cDNA Expression Array (Clontech) to determined bladder gene modulation in response to LPS, SP, or antigen.

Results: Gene cluster analysis indicates genes that were commonly up-regulated in response to LPS, SP, and antigen stimulation. The following genes were up-regulated by at least one of the stimulus: nerve growth factor beta, IL-1, IL-4, IL-6, IL-12, TGF beta, TIMP-3, VEGF, GRAF1, TNF55, TNFR-1, angiotensin converting enzyme, interleukin converting enzyme, ICAM-1, VCAM-1, VEGF, and GCSF.

Conclusion: The combination of morphological changes and genomic alterations represents a powerful approach for to study basic mechanisms of inflammation. A better understanding of the molecular mechanisms leading to cystitis will permit the design of new therapeutic strategies to decrease inflammation.

Hemidesmosomes in Bladder Epithelial Cells

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Interaction between cells and the extracellular matrix has an impact on the regulation of a variety of cellular functions including adhesion, motility, gene expression, differentiation and proliferation. Our research on this topic has focused on analysis of the structure, assembly and functions of a matrix junction called the hemidesmosome via which bladder epithelial cells tether to specific extracellular matrix proteins in the basement membrane zone. We have used cell cultures of bladder cells, namely rat 804G cells, as our model system.

Structure: Using antibodies, molecular genetic approaches and yeast two hybrid assays, we have shown that hemidesmosomes in 804G cells possess an integrin heterodimer composed of a6 and b4 subunits. The a6b4 integrin complex binds to the G domain of laminin-5 in the extracellular matrix as well as to a type II transmembrane element of the hemidesmosome termed BP180. In the cytoplasmic plaque of the hemidesmosome, a6b4 integrin binds plectin while BP180 interacts with a protein called BP230. BP230 and plectin mediate interaction of the keratin cytoskeleton with hemidesmosomes.

Assembly: Antibodies against the extracellular domain of the b4 integrin subunit inhibit hemidesmosome assembly in 804G cells, indicating that the a6b4 integrin plays a crucial role in assembly of hemidesmosomes. Laminin-5 undergoes precise proteolytic processing in the extracellular milieu of 804G cells. Prior to processing laminin-5 promotes migration of epithelial cells. However, once processed, it becomes competent to drive hemidesmosome assembly in epithelial cells in an a6b4 integrin-dependent manner.

Functions: The major function of a hemidesmosome is as a site of stable adherence. Epithelial cells that are plated onto laminin-5 derived from 804G cells resist detachment even when subject to forces of more than up to 90 dynes/cm2. In addition, components of the hemidesmosome, including laminin-5, are involved in signal transduction that impact cell proliferation. For example, antibodies against the G domain of laminin-5 regulate 804G cell growth via a signaling pathway involving mitogen-activated protein kinase. These topics will be explored in the presentation with an emphasis on dynamic aspects of hemidesmosome assembly and function.

Stretch-Regulated Exocytosis of Discoidal Vesicles in Urinary Bladder Epithelium

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The urinary bladder accommodates large increases in urine volume during filling while maintaining a barrier to the toxic substances present in the urine. When the bladder is empty, the superficial cells that line the lumen contain a population of sub-apical cytoplasmic vesicles. It is hypothesized that during filling the vesicles fuse with the apical membrane in order to increase apical surface area. To test this hypothesis, we mounted rabbit bladders in modified Ussing chambers and subjected the tissue to hydrostatic stretch. Apical membrane capacitance (where 1μF = 1cm^2) was measured during stretch to estimate changes in the apical surface area. The capacitance rapidly increased by 15% within the first 20 minutes of stretch and gradually increased an additional 40% above control values over a 5-hour period. Stereological measurements confirmed that after 5 hours apical membrane surface area increased from 2905 ± 453 μm^2 in controls to 4379 ± 844 μm^2 in stretched samples. Biochemical analysis revealed that the amount of Uroplakin III (a membrane/vesicle-associated protein) at the apical surface increased 8-fold after stretch, with a concomitant decrease in vesicle surface area from 7234 ±1070 μm2 to 728 ± 194 μm^2. Intracellular levels of cAMP were elevated 200% within 15 minutes of stretch. Treatment of either control or stretched cells with 10μM forskolin increased the apical surface area above control values to 6557 ± 1420 μm^2 and 7382 ± 1468 μm^2, respectively, and changes in capacitance were blocked by treatment with the PKA inhibitor H-89. Our results are consistent with the vesicle fusion hypothesis and indicate that this fusion is regulated, in part, by changes in cAMP.

Plasticity of the Urothelial Phenotype: Effects of Gastro-Intestinal Mesenchyme/ Stroma and Implications for Urinary Tract Reconstruction

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We have shown that stromal-epithelial interactions are necessary for the development of the bladder. Specifically, without urothelium, bladder smooth muscle does not develop. The present study continues the theme that reciprocal cell-cell signaling occurs within the urinary tract. Herein, we tests the hypothesis that heterotypic stromal-epithelial interactions cause phenotypic changes in urothelium. The rational for the experimental design is to simulate heterotypic stromal–epithelial interactions that are created at the anastomotic site of intestinal-bladder augmentations and internal urinary diversions where the urothelium is in direct contact with the gastrointestinal tract tissues.

Tissue recombination experiments were performed by combining rat and mouse rectal mesenchyme/stroma with urothelium from embryonic, newborn and adult mice or rats. Analysis was performed to detect expression of proteins specific to urothelium (uroplakins, cytokeratin 7, 14, and 19) and rectal epithelium (Periodic Acid-Schiff (PAS)).

The phenotype of both mouse and rat urothelium was changed to a glandular morphology under the influence of rectal mesenchyme. Immunohistochemical staining revealed a loss of the urothelial specific uroplakins and cytokeratins 7, 14 and 19 (characteristic of urothelium). Histologic analysis revealed the presence of mucin secreting glandular structures, which stained positive for PAS. The urothelial transdifferentiation into glandular epithelium was not a function of epithelial age and occurred in the embryonic, newborn and adult urothelium. Likewise, rectal mesenchyme from embryonic, neonatal and adult animals was able to induce glandular differentiation in bladder epithelium.

Urothelium exhibits the plasticity to change into an intestinal like epithelium as a result of mesenchymal/stromal stimulation from the gastrointestinal tract. This experimental result is germane to heterotypic stromal-epithelial interactions that are created in patients with urinary tract reconstructions (intestinal augmentations, demucosalized urothelial lined bladder patches and internal urinary diversion such as ureterosigmoidostomies). We propose that heterotypic stromal-epithelial interactions may play a role in determining histodifferentiation of urothelial cells at the anastomotic site between bowel and bladder tissue in patients with gastro-intestinal urothelial reconstructions.

Antiproliferative Factor (APF), Heparin-Binding Epidermal Growth Factor-Like Growth Factor (HB-EGF), and Epidermal Growth Factor (EGF)—Sensitive and Specific Urine Markers for Interstitial Cystitis (IC)

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We previously determined that the urine of IC patients specifically contains a factor (APF) that inhibits primary bladder epithelial cell proliferation, and that it has significantly decreased HB-EGF and increased EGF levels compared to urine from asymptomatic controls and patients with bacterial cystitis. We sought to confirm the specificity of these findings for IC using a larger patient population, including control patients with a variety of both inflammatory and noninflammatory urogenital disorders.

Clean catch urine specimens were collected from symptomatic IC patients, asymptomatic controls without bladder disease, and patients with acute bacterial cystitis, vulvovaginitis, chronic nonbacterial prostatitis, overactive bladder, hematuria, stress incontinence, neurogenic bladder, benign prostatic hyperplasia, bladder or pelvic pain without voiding symptoms, bladder cancer, prostate cancer, or miscellaneous diagnoses including anatomic disorders. APF activity was determined by ^{3}H-thymidine incorporation into primary normal adult human bladder epithelial cells. HB-EGF and EGF levels were determined by ELISA.

APF activity was present significantly more often in IC than control urine specimens ($p<0.01$ for IC vs. any control group; sensitivity=94%, specificity=95%, PPV=88%, NPV=95% for IC vs. all controls). HB-EGF levels were also significantly lower and EGF levels significantly higher in IC urine than in specimens from any of the control groups ($p<0.01$).

These findings confirm the utility of APF, HB-EGF and EGF as markers for IC. Understanding the reasons for altered levels of these markers may lead to understanding the pathogenesis of this disorder.

Positive and Negative Regulators of Human Urothelial Cell Proliferation

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The urothelium exhibits one of the slowest turnover rates among mammalian epithelia - yet it can rapidly regenerate during development or in response to injury. Normal urothelial cell proliferation is therefore a tightly regulated process. An *in vitro* model system was developed to critically examine the involvement of transmembrane receptor tyrosine kinases in this process. Primary cultures of urothelial cells derived from renal pelvis, ureter, or urinary bladder were used in these studies.

Two members of the fibroblast growth factor (FGF) family of polypeptides were identified as positive regulators of urothelial proliferation. FGF-7 and FGF-10 were cloned from human urinary bladder and expressed as biologically active proteins in the cytoplasm of *Escherichia coli.* Each recombinant (r) protein was engineered to contain a C-terminal histidine hexamer. Both rFGF7-His and rFGF10-His bound heparin with similar affinities as their wild-type counterparts. Each polypeptide stimulated the incorporation of [^{3}H]-thymidine into the cellular DNA of primary cultures of human urothelial cells *in vitro*. Stimulation of DNA synthesis was concentration-dependent. Analyses of cellular lysates indicate that the 92 kDa FGFR2IIIb transmembrane receptor kinase is involved for transducing the rFGF7-His signal but not necessarily the rFGF10-His signal. Immunoprecipitations of FGFR2IIIb:rFGF7/10-His complexes have revealed the existence of a novel 72 kDa polypeptide from urothelial cells cultured from renal pelvis or from urinary bladder.

Secreted Protein Acidic and Rich in Cysteine (SPARC) was identified as a negative regulator of urothelial cell proliferation. Recombinant SPARC inhibited urothelial cell DNA synthesis under the same conditions in which recombinant FGF was mitogenic. Urothelial-derived SPARC was found to be secreted into the abluminal compartment. It is hypothesized that constituently-expressed levels of SPARC function to maintain the urothelium in a quiescent state *in vivo*. A proposed mechanism to achieve this quiescence is by abrogating the binding of FGF to its cognate receptor on the basolateral face of the urothelial cell plasma membrane.

Role of Prostglandins in Bladder Smooth Muscle Cell Gene Expression

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Bladder smooth muscle cells (SMC) play an important role in the progression of bladder deterioration after outlet obstruction. Altered mechanical environment within the obstructed bladder may be a critical factor in triggering the pathological bladder SMC responses. We demonstrated previously that bladder obstruction stimulated the expression of inducible cyclooxygenase isoform, COX-2, in bladder SMC and that this occurred by increased mechanical stretch. COX-2 regulates a key rate-limiting step in prostaglandin biosynthesis. The exact function of COX-2 gene activation in bladder SMC is not known, but one possible role may be that COX-2 mediated prostaglandins regulate the expression of other down-stream genes. Peroxisome proliferator-activated receptor g (PPARg) is a member of nuclear receptors that plays a key role in adipocyte differentiation and inflammation. It has been demonstrated recently that a prostaglandin J2 metabolite, 15-deoxy-$D^{12,14}$-prostaglandin J2 (15d-PGJ2), is a potent endogenous ligand for PPARg.

We investigated whether this paradigm of 15d-PGJ2/ PPARg nuclear regulation exists in bladder SMC in the setting of outlet obstruction. By Western immunoblot analysis, we were able to detect the presence of PPARg protein in the rat bladder SMC *in vivo*. Furthermore, with chronic outlet obstruction, there was a specific pattern of alteration in PPARg protein levels. When the partially obstructed rat bladders were assessed up to 6 weeks, COX-2 gene activation was seen only during the first 24 hours, while PPARg levels transiently declined between 1 and 3 days of obstruction and gradually increased thereafter. Although the exact functional significance of these changes is not known, such pattern of PPARg levels suggests a possible biological role.

We also studied the role of 15d-PGJ2/ PPARg nuclear regulation pathway in the stretch-stimulated expression of COX-2 in cultured bladder SMC. Primary rat bladder SMC were grown on collagen I coated silicone membranes and stimulated with cyclical stretch and relaxation (0.1 Hz, 20% elongation, FX-3000, Flexcercell Corp.) in serum-deficient media. When the cells were stretched in the presence of 15d-PGJ2, there were dose-specific alterations in the stretch-induced expression of COX-2. At 1 to 5 m M 15d-PGJ2, there was a synergistic super-induction of COX-2 level after stretch stimulation. At 10 m M, however, 15d-PGJ2 significantly attenuated COX-2 expression. This super-induction response by low dose 15d-PGJ2 was completely suppressed by p38/SAPK inhibitor, SB203580 (10 m M), and was unaffected by ERK/MAPK inhibitor, PD98059 (30 m M). By electrophoretic mobility shift assay, we also demonstrated that stretch-induced AP-1/DNA binding was similarly affected by 15d-PGJ2 in a dose-specific manner. A synthetic thiazolidinedione (TZD) compound known to activate PPARg, troglitazone (20 m M), suppressed the stretch-stimulated COX-2 expression, while a PPARa specific ligand, WY-14643 (10-100 m M), had no effect. Interestingly, when the cells were stretched for 24 hours in the presence of COX-2 inhibitor, NS-398 (30 m M), PPARg protein levels decreased in bladder SMC.

Collectively, these findings suggest that PPARg may be a novel transcriptional regulator for bladder SMC in the setting of outlet obstruction. Furthermore, 15d-PGJ2/PPARg pathway may be a potential mechanism by which COX-2 mediated prostaglandins play a role in the nuclear regulation of down-stream target genes in bladder SMC.

Uropathogenic *E. coli:* Interactions With Bladder Epithelium

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Uropathogenic strains of *Escherichia coli* are the primary causative agents of cystitis and upper urinary tract infections. Filamentous surface adhesive organelles called type 1 pili are encoded by virtually all strains of uropathogenic *E. coli* and are assembled via the chaperone/usher pathway. The periplasmic chaperone is comprised of two immunoglobulin (Ig-like) domains. It forms chaperone-subunit complexes that are targeted to the outer membrane usher where the subunits are assembled into the pilus. Using high-resolution electron microscopy, we have shown that the adhesive tips of type 1 pili, which contain the adhesin molecule FimH, can interact directly with host receptors on the lumenal surface of the bladder epithelium. FimH is comprised of a pilin domain and a receptor binding domain based on X-ray crystallography. The pilin domain of FimH and the single domain of the pilus subunits have Ig folds that lack the seventh (C-terminal) b-strand present in canonical Ig folds. The absence of this strand produces a deep groove along the surface of the pilin domain. In the chaperone-subunit complexes, the chaperone contributes its G1 b-strand to complete the Ig fold of the subunit by occupying the groove, an interaction termed donor strand complementation. During pilus biogenesis, the N-terminal extension of one subunit is thought to displace the chaperone G1 strand and insert into the groove of the neighboring subunit via a mechanism termed donor strand exchange. Thus, every subunit in a pilus completes the Ig fold of its neighboring subunit. Attached pili mediated intimate contact of the bacteria with the bladder surface, which is coated with hexagonal arrays of integral membrane proteins known as uroplakins. Bacterial attachment facilitated by FimH, in addition to as of yet unidentified bacterial factor(s), triggered the exfoliation of host bladder epithelial cells as part of an innate host defense system. Exfoliation occurred via an apoptosis-like mechanism requiring caspase activation and involving host DNA fragmentation. Using various biochemical, genetic, and microscopic assays, we have found that the type 1 pilus adhesin can mediate the internalization of bacteria into bladder epithelial cells. Internalized uropathogenic strains of *E. coli* can replicate and microscopic studies of infected mouse bladders have indicated that the intracellular bacteria can eventually break out of infected host cells and colonize surrounding tissue. Fluxing in and out of bladder epithelial cells may provide a means for uropathogenic *E. coli* to resist clearance from the bladder by both innate and adaptive host defenses and may also facilitate the spread of uropathogens within the urinary tract. These studies are revealing the structural basis and the consequences of host-pathogen interactions that are required for persistent urinary tract infections.

RTX

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Intravesical Peppers: The normal sensations of bladder filling appear to be mediated by small myelinated A-delta fibers. However, C-fiber afferents, which are small and unmyelinated, have very high mechanical thresholds and do not respond to even high levels of intravesical pressure. C-fibers are activated by noxious chemical irritation or by cold. In the irritated state, these fibers become responsive to low pressure bladder distension like mechanoreceptive A-delta fibers. C-fibers, therefore, are normally "silent" and appear to have a specific function, i.e. signaling of inflammatory or noxious events in the bladder (Chancellor and de Groat 1999).

The vanilloids, capsaicin and resiniferatoxin, activate nociceptive sensory nerve fibers through an ion channel, recently discovered by Caterina et al. (1997), known as vanilloid receptor subtype 1 (VR1). This receptor is a nonselective cation channel, and is activated by increases in temperature to within the noxious range and by protons, suggesting that it functions as a transducer of painful thermal stimuli and acidity *in vivo.* When activated the channel opens, allowing an influx of calcium and sodium ions that depolarizes the nociceptive afferent terminals, initiating a nerve impulse that travels through the dorsal root ganglion into the central nervous system. Noxious temperature uses the same elements, which explains why the mouth feels hot when eating chili peppers (Clapham, 1997). Previously called the capsaicin receptor, VR1 has been localized in the spinal cord, dorsal root ganglia and visceral organs, including the bladder, urethra and colon. Activation of VR1 results in spike-like currents (Liu and Simon, 1996), and selectively excite and subsequently desensitize C-fibers. Capsaicin desensitization is defined as long lasting, reversible suppression of sensory neuron activity (Craft et al., 1995).

RTX for Interstitial Cystitis (IC): Lazzeri et al. (2000) recently presented prospective randomized result of intravesical RTX in IC patients from Italy. In 18 patients with bladder hypersensitivity, there was a significant improvement in urinary frequency/24 hours, nocturia, and pain scale recording between RTX (10 nM) vs placebo (saline) treatment patients after 30 days and partial persistent effect after 90 days. We feel it would be a priority to study intravesical RTX in IC patients in an FDA-approved protocol in the USA.

Gene Therapy for IC: We believe that the field of urology, along with all specialties of medicine, is on the brink of a revolution called molecular medicine. While traditional medicine treats symptoms, gene therapy addresses the deficiency that causes the symptoms. With improved understanding of the human genome and evolving techniques to construct gene therapy vectors that manipulate our genetics, the way we practice medicine will be forever changed.

We propose a revolutionary concept in the treatment of IC and visceral bladder pain independent of etiology. We hypothesize that targeted and localized expression of enkephalin in the nerves that innervate the bladder by gene transfer can treat bladder pain. b-Galactosidase staining was used to detect lacZ expression in female Sprague- Dawley (250-300 g) rat bladder and L6 dorsal root ganglia (DRG) after

bladder injection with SHZ virus (HSV-1 with lacZ insert, 5 X 10^8 pfu). At 7, 14 and 30 days after bladder inoculation with SHPE (HSV-1 with preproenkephalin cDNA insert, 5 X 10^8 pfu), bladder tissue and DRG (L4, L6, S1) transgene levels were quantified with PCR techniques using primers specific for human preproenkephalin gene (PPE). L4 was chosen for the minimal afferent innervation from the bladder. Preliminary CMG experiments were performed in untreated SD rats after administration of intrathecal met-enkephalin and intrathecal naloxone. Cystometric studies under urethane anesthesia were also done one week after injection with SHPE (n=10) or SHZ as control (n=10). Continuous intravesical capsaicin (15uM) infusion was used as a bladder irritant, while i.m. naloxone (0.5 mg/kg) was used as an opioid antagonist.

Beta-galactosidase staining was observed in bladder and L6 DRG 1 week after bladder injection with SHZ. Small and medium-sized cell bodies had LacZ activity, with a paucity of staining in larger cell bodies. Quantitative PCR after bladder injection with SHPE also demon-strated preproenkephalin transgene expression in L6>S1>>L4 sensory ganglia at all time points, consistent with afferent innervation of the rat urinary bladder. An early peak level of transgene was observed at 7 days in the bladder, while a later increased level seen in L6 DRG at 30 days. Cystometric studies showed that intrathecal met-enkephalin (10 ug) blocks the acute effects of intravesically applied capsaicin (15uM) in untreated SD rats. This effect was antagonized by i.t. naloxone (4ug). SHPE injected rats demonstrated a significant increased intercontraction interval (ICI, minutes) at baseline saline CMG versus SHZ rats (15.1±3.0 and 6.1±0.97, p=0.009). The reduction in the ICI induced by intravesical injection of capsaicin (15mM) was significantly smaller in SHPE rats than SHZ rats (24% versus 35% change, p=0.04). However this difference in response to capsaicin in the ICI was occluded by i.m. naloxone (0.5 mg/kg), which suggests that enkephalinergic mechanisms were upregulated to suppress capsaicin-induced bladder hyperactivity in SHPE-injected animals.

The preproenkephalin gene could be transferred and maintained in the urinary bladder and bladder afferent nerves using HSV vectors. We demonstrated proof of concept that gene therapy for bladder pain is not only feasible but can suppress nociceptive responses induced by bladder irritation. This technique of gene transfer may be useful for treating IC and other types of visceral pain.

Conclusions: There is a revolution starting in urology. This revolution involves how we think about and treat IC. The concept of intravesical capsaicin and resiniferatoxin promises target-specific and long-lasting therapy in the near future. We also have speculated about the farther horizon of molecular medicine, through which gene therapy may help all types of bladder and genitourinary tract pain.

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Interstitial Cystitis in Children—Not a Rare Entity

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Schuster & Lewis, MDSC, Joliet, IL

Forty-nine children who presented with voiding dysfunction were evaluated in a systemic manner consisting of a detailed history, focused physical examination, urinalysis, voiding diary, residual urine, and a cystoscopy and hydraulic distention of their bladder. Twenty-six children completed the evaluation and were available for study. Eighteen of the 26 (69%) also had urodynamics performed.

Modifications of the National Institutes of Health minimal criteria for interstitial cystitis were made as follows to accommodate children. A frequency of urination, while awake, less than eight times per day was defined as voiding less than 1/3 of their estimated bladder capacity per void as measured from their voiding diary. Absence of nocturia: included bed wetting. Bladder capacity greater than 350 ml on awake cystometry was defined as less than 80% of estimated bladder capacity (estimated bladder capacity calculated as age/2 + 6 in ounces). Absence of an intense urge to void with the bladder filled to 150 ml of water during cystometry was defined as an intense urge to void at less than 1/3 bladder capacity.

Twelve children failed to meet NIH criteria. Three children failed NIH criteria because they had minimal or no glomerulations noted on cystoscopy. Nine children with glomerulations on cystoscopy failed NIH criteria. One child failed because her maximum voided volume exceeded 80% of her expected maximum volume. Three children failed because they did not have urodynamics performed and did not have pain with bladder filling. Three children failed because they did not have an intense desire to void at less than 1/3 estimated bladder capacity on cystometry. Two children failed because they exceeded 80% of estimated bladder capacity on cystometry.

Fourteen of the 26 (54%) met the NIH minimum criteria for interstitial cystitis. Interstitial cystitis in children is not as “rare” as the literature would suggest.

Interstitial Cystitis in India: Lessons

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Objective: To study the interstitial cystitis (IC) patient population characteristics, diagnostic methodology, treatment planning and results of management in single center situated in western India and to derive important lessons.

Material and Method: From April '93 to August 2000, 48 patients were diagnosed to be suffering from IC. 15 were males and 33 females ages from 19 to 63. All the patients were suffering from chronic frequency, urgency and dysuria syndrome (FUDS). All these patients had undergone CBC, urine routine and culture, X-ray KUB and USG KUB study and all were essentially normal. Cystoscopy was done under anaesthesia and bladder was filled by gravity with saline till capacity with reservoir height being 80 cms and drained. Bladder was distended again till capacity and this distension was maintained for 2-3 minutes. Bladder was drained again and cold cup biopsy was taken. Few patients were treated with amytryptaline. In one patient intravesical DMSO was instilled. In same patient intravesical BCG was instilled later on. In two patients with intractable disease and small capacity bladder augmentation cystoplasty was done.

Result: Out of 48 patients 23 (50%) are having no symptoms after treatment. These include two patients of surgical management. 14 (30%) have shown no improvement. Rest (20%) are better than before. Experience of intravesical BCG in single patient is very bad.

Conclusion:

1. IC was more common in males than thought.
2. All patients of FUDS should be subjected to cystoscopy under anesthesia and HD as it has a prolonged therapeutic value in 70% of cases.
3. Limited HD of 2-3 minutes is perhaps better than prolonged HD.
4. Glomerulations developed in most cases after first evacuation rather than on distension.

Observations on the Presentation, Diagnosis, and Management of Interstitial Cystitis in Men

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A series of 52 men with the diagnosis of interstitial cystitis established utilizing the NIDDK criteria is presented for review. The responses to therapy are presented.

The mean age of diagnosis was 46 years of age. Ninety-five percent of patients are white and 5 percent are Native American. The average time from the development of persistent symptomatology to the established diagnosis was 26 months. The most common referral diagnoses to our clinic were prostatitis, BPH, epididymitis, and recurrent urinary tract infections. The most common symptoms in decreasing order were suprapubic discomfort, urinary frequency, dysuria, testicular pain, and sexual dysfunction.

Cystoscopy with hydraulic distention of the bladder was performed in all patients. All patients demonstrated diffuse glomerulations. Twelve percent of patients demonstrated severe ulcerations. Urodynamic studies revealed low-volume, low-pressure voiding patterns. All urinary cultures were negative.

Intravesical therapy historically was successful in controlling symptomatology in 70% of patients with greater than 70% failure rate over time. Multi-drug oral therapy produced greater than a 75% improvement in symptomatology in greater than 80% of the patient population.