Agenda

Please note that all PI presentations should be 20 minutes in duration with an additional 5 minutes for questions and answers

	DAY 1: WEDNESDAY, OCTOBER 20, 2004
7:30 – 8:30 am	**Registration and Continental Breakfast**
8:30 – 8:45 am	Welcome and Opening Remarks Chris Mullins, PhD, NIDDK, NIH
	Session I: Pelvic Pain and Neurobiology (8:45 am – 12:00 pm) Chair: Margaret A.Vizzard, PhD
8:45 am	Margaret A.Vizzard, PhD, University of Vermont and State Agricultural College *Neurotrophic Mechanisms in LUT Plasticity with Cystitis*
9:10 am	Michael A. Pezzone, MD, PhD, University of Pittsburgh *Neurogenic Pathogenesis of Interstitial Cystitis*
9:35 am	Ursula Wesselmann, MD, Johns Hopkins University School of Medicine *Pain and Interstitial Cystitis*
10:00 am	Alan Randich, PhD, University of Alabama at Birmingham *Effects of Early-In-Life Bladder Stimulation on Adults*
10:25 – 10:45 am	**Break**
10:45 am	Jay Reeder, PhD, Rochester Medical Center (for Edward M. Schwarz, PhD) *PACAP Overexpression and Voiding Function*
11:10 am	Mary-Patricia Fitzgerald, MD, Loyola University in Chicago *Perceptions During Bladder Filling, Cutaneous Current Perception Thresholds and Response to Repetitive Cutaneous Stimulation in Painful Bladder Syndrome/Interstitial Cystitis*

11:35 am	**Session I Panel Discussion: Advances, Needs and Future Directions**
12:00 – 1:00 pm	**Lunch** (on your own)
1:00 – 1:45 pm	**Guest Speaker: Translational Research** Susan Keay, MD, PhD Professor of Medicine, Division of Infectious Diseases University of Maryland Veterans Affairs Medical Center *Complete Characterization of an Antiproliferative Factor from the Urine of Interstitial Cystitis Patients*
	Session II: Inflammation and Immunobiology (1:45 pm – 3:45 pm) Chair: David J. Klumpp, PhD
1:45 pm	David J. Klumpp, PhD, Northwestern University *Mast Cell Migration During PRV-Induced Neurogenic Cystitis*
2:10 pm	Jane McHowat, PhD, Saint Louis University *Tryptase Activation of Calcium-Independent Phospholipase A_2 in Human Bladder Endothelial Cells*
2:35 pm	Yi Luo, MD, PhD, University of Iowa *Development of Urinary Bladder Autoimmune Cystitis Model (Progress Report)*
3:00 pm	Raymond Rackley, MD, The Cleveland Clinic *The Role of the NF-κB Signaling Pathway in the Pathogenesis of Interstitial Cystitis*
3:25 pm	**Session II Panel Discussion: Advances, Needs and Future Directions**
3:45 – 4:00 pm	**Break**
	Session III: Urothelial Cell Biology and Barrier Function (4:00 pm – 6:00 pm) Chair: Jennifer Southgate, PhD
4:00 pm	Jennifer Southgate, PhD, University of York *Urothelial Barrier Function and Interstitial Cystitis*

4:25 pm	John W. Hanrahan, PhD and Fernando Cervero, MD, PhD, DSc, McGill University *Epithelially-Derived Factors in Interstitial Cystitis*
4:50 pm	Monica Liebert, PhD, University of Michigan *IL8 Expression in Urothelial Differentiation and Interstitial Cystitis*
5:15 pm	Tung-Tien H. Sun, PhD, New York University *Regulation of Urothelial Growth and Differentiation*
5:40 pm	**Session III Panel Discussion: Advances, Needs and Future Directions**
6:00 – 8:00 pm	**Poster Session/Networking**
	DAY 2: THURSDAY, OCTOBER 21, 2004
7:45 – 8:30 am	**Continental Breakfast**
	Session IV: Genetics, Genomics and Proteomics (8:30 am – 10:30 am) Chair: Brian C. Liu, PhD
8:30 am	Brian C. Liu, PhD, Brigham and Women's Hospital *Proteomics Approaches to Interstitial Cystitis*
8:55 am	Fernando de Miguel, PhD, University of Pittsburgh *Proteomics Characterizations of Interstitial Cystitis Bladder: Searching for New Markers*
9:20 am	Ricardo Saban, DVM, PhD, University of Oklahoma Health Sciences Center *Mouse Bladder Transcriptome in Response to BCG Therapy*
9:45 am	John W. Warren, MD, University of Maryland *Two Studies:* *1. Events Preceding Interstitial Cystitis (EPIC)* *2. Maryland Genetics of Interstitial Cystitis (MaGIC)*

10:10 am	**Session IV Panel Discussion: Advances, Needs and Future Directions**
10:30 -10:50 am	**Break**
	Session V: Etiology, Diagnosis and Treatment for Interstitial Cystitis (10:50 am – 12:55 pm) Chair: Jordan Dimitrakov, MD, PhD
10:50 am	Jordan Dimitrakov, MD, PhD, Children's Hospital/Harvard Medical School *Diagnostic Challenges in Interstitial Cystitis (and Male Chronic Pelvic Pain Syndrome)*
11:15 am	Dale E. Bjorling, DVM, University of Wisconsin-Madison *Neurovascular Responses to Cystitis*
11:40 am	Fernando de Miguel, PhD, University of Pittsburgh (for Naoki Yoshimura, MD, PhD) *Bladder Pain Gene Therapy with Opioid cDNA*
12:05 pm	Vinata B. Lokeshwar, PhD, University of Miami *Urinary Uronate and Sulfated Glyacosaminoglycan Levels:* *Markers for Interstitial Cystitis Severity*
12:30 pm	**Session V Panel Discussion: Advances, Needs and Future Directions**
12:55 – 2:00 pm	**Lunch** (on your own)
2:00 – 3:00 pm	**Joint Meeting of the Interstitial Cystitis Basic Investigators' Group and the Interstitial Cystitis Clinical Research Network (ICCRN)** Guest Speaker: C.A. Tony Buffington, DVM, PhD Professor of Veterinary Clinical Sciences Ohio State University *Neuroendocrine Issues in Interstitial Cystitis?*
3:00 pm	**Adjournment for Interstitial Cystitis Basic Science Investigators' Meeting** **Continue ICCRN Meeting in closed session**

Organizing Committee

Chris Mullins, PhD
National Institute of Diabetes
and Digestive and Kidney Diseases, NIH

Leroy Nyberg, MD, PhD
National Institute of Diabetes
and Digestive and Kidney Diseases, NIH

Jordan Dimitrakov, MD, PhD
Children's Hospital
Harvard Medical School

David Klumpp, PhD
Northwestern University

Brian C. Liu, PhD
Brigham and Women's Hospital

Jennifer Southgate, PhD
University of York

Margaret A. Vizzard, PhD
University of Vermont
State Agricultural College

Neurovascular Response to Cystitis

D.E. Bjorling, S. Guerios, L.M. Janda,
W.J. Hopkins, J. Elkahwaji, Z-Y Wang

University of Wisconsin, Madison, WI

The long-range goals of this research are to characterize changes in sensory innervation and vasculature of the bladder that occur in response to acute and chronic cystitis in a mouse model and further to identify clusters of genes that render mice more or less sensitive to cystitis. We have investigated the effects of low dose (15-35 mg) intraperitoneal cyclophosphamide (CYP) on bladder morphology and thermal and mechanical sensitivity of the hind paw in mice. Low-dose CYP failed to induce distinct histological changes in the bladder, but the threshold of sensitivity to mechanical stimuli was significantly reduced 4 hours after CYP treatment compared to saline-treated controls. Sensitivity to mechanical stimuli was enhanced up to 48 hours after a single injection of CYP. CYP injection had no effect on the threshold for thermal stimuli applied to the hind paw.

To further pursue the goals of this research, a controlled method for inducing cystitis by intravesical instillation of acrolein (the active metabolite of cyclophosphamide) has been developed. We found that low doses of acrolein (2 μg) instilled into the bladder of C57BL/BN mice causes a significant increase in bladder weight 4 hours after instillation in the absence of obvious histological changes. Increasing doses of acrolein up to 100 μg cause further increases in bladder weight. Dose higher than this cause submaximal increases in bladder weight, and this appears to be due to loss of structural integrity of the bladder.

These observations indicate the occurrence of significant changes in innervation and vascular permeability of the bladder at doses of CYP or acrolein (the active metabolite of CYP) that do not stimulate overt changes in morphology. The use of low concentrations of irritant compounds and the sensitivity of these models will allow us to critically assess differences in response to these stimuli among various strains of mice. The chromosomal site containing a gene associated with a quantitative phenotype such as response to a given stimulus is regarded as a quantitative trait locus (QTL), and it is possible to estimate the number of QTL for a given phenotype. Other investigators have reported strain differences in the susceptibility of mice to CYP injection, but these experiments were performed using relatively high doses (150-300 mg/kg) of CYP. We anticipate that our models will allow us to identify genes associated with relative sensitivity to bladder irritation, and we will then evaluate the presence and activity of corresponding genes in patients with bladder disorders such as interstitial cystitis (IC).

Proteomic Characterization of Interstitial Cystitis Bladder: Searching for New Markers

Fernando de Miguel, Suzy van Le, Yukio Hayashi, Naoki Yoshimura, Michael B. Chancellor

Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, PA

Objectives:
Interstitial cystitis (IC) is a painful bladder syndrome of unknown etiology, characterized by chronic pelvic pain and urinary frequency and urgency. Diagnosis of IC is primarily based on symptoms, as there are no currently available blood or urine tests due to the lack of demonstrated biological markers. Nuclear and cytoplasmic proteins have been shown to be detectable in a number of diseases, both malignant and non-malignant. Therefore, we propose to use a proteomic approach to identify specific new markers related to IC in two rat chronic cystitis models.

Methods:
Two rat models of chronic cystitis were utilized: a) A one time intravesical instillation of 0.2 ml 0.4N Hydrochloric Acid (HCl), and b) Intraperitoneal injections of 75 mg/kg cyclophosphamide (CYP) every third day for 14 days. Whole bladders from both IC models, as well as controls, were collected and snap frozen. The NE-PER Kit (Pierce Biotechnology) was used to isolate both nuclear and cytoplasmic proteins from each tissue and then run on the Protean® IEF System with immobilized pH gradients (BioRad) for 2-Dimensional (2D) SDS-PAGE gel analysis. After electrophoresis, the gels were initially stained with Silver Stain Plus (BioRad) and analyzed. Differential protein spots were compared between the normal and each IC model. Once different spots were identified, samples were repeated on the 2D gel system and subsequently stained with Sypro Ruby (Invitrogen). Gels were placed in a home-built gel imaging device (University of Pittsburgh Genetics and Proteomics Core Laboratories) with an integral gel cutting tool. Gel plugs were excised using an automated picker into a microtiter plate containing 1% acetic acid. The gel plugs were then sequenced by peptide mass fingerprinting (PMF) at the Core facilities.

Results:
The three methods employed for gel analysis (silver stain, Sypro Ruby, or DIGE with Cy3/Cy5 labeling) yielded useful images. We found little or no difference between the normal and the CYP tissues on 2D gel patterns. However, many differences were seen between the normal and HCl tissues. Grossly, six spots that were apparent in nuclear extracts of normal bladders were not present in HCl gels, and nine spots present in nuclear extracts of HCl bladders were not present in normal nuclear extracts. Moreover, three spots from cytosolic extracts of normal bladders were not present in HCl cytosolic extracts, whereas two spots from HCl cytosolic extracts were not seen in cytosolic normal extracts. Currently, three nuclear proteins have been identified by PMF: smooth muscle 22/transgelin (downregulated in nuclear extracts in HCl bladder), Ras suppressor protein-1 (RSU-1, downregulated in nuclear extracts in HCl bladder), and GAPDH (downregulated in nuclear extracts in HCl bladder).

Conclusions:
The choice of detection method for 2D gels depends on the needs of the user and the protein ID method. Using 2D-gels we have identified several proteins that could be involved in the pathogenesis of IC, and could be potential markers/molecular targets of drug therapy.

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Diagnostic Challenges in Interstitial Cystitis (and Male Chronic Pelvic Pain Syndrome)

Jordan D. Dimitrakov

Harvard Urological Diseases Research Center, Children's Hospital/Harvard Medical School, Boston, MA

A major obstacle in undertaking research on interstitial cystitis (IC) is the absence of any kind of molecular "handle" onto the disease. Numerous competing theories about the origin—and even the definition—of interstitial cystitis abound. Studies on tissue are difficult because tissue procurement from affected persons is difficult and limited. No accepted animal model of the disease exists. The finding that decreased level of HB-EGF in urine is a highly sensitive and specific marker of interstitial cystitis provide a tantalizing hint into the etiology of this disease, which to date remains completely obscure. Our research efforts have been directed at the identification and categorization of the constituents of the urinary proteome in IC patients and the manner in which these proteins interact with other urinary proteins and with molecules present in normal and disease (IC) states *(the urinary interactome)*. Moreover, based on the urinary interactome, we are attempting identification and validation of several surrogate urine and tissue markers that correlate with stage, activity or rate of progression, and/or complications of IC as compared with controls. In our initial studies, we have identified and validated several candidate biomarkers and measured their levels during IC flare-ups and remissions. We have constructed several microelectromechanical (MEMS) devices for intravesical as well as non-invasive (percutaneous) recording of bladder smooth muscle contractions and correlation with disease (IC) activity. We also compiled a panel of candidate biomarkers and incorporated it into a nano-sized mass spectrometry microchip ["IC mini-mass spec" (ICMMS)]. Several experiments with nanoparticles – nanotubes and nanovesicles – for targeted gene- and drug-delivery to IC bladders are also under way. Our results underscore the importance of "theranostics" – the combination of therapy-driven diagnostic approaches – as the key to the effective management of IC.

Perceptions During Bladder Filling, Cutaneous Current Perception Thresholds and Response to Repetitive Cutaneous Stimulation in Painful Bladder Syndrome/Interstitial Cystitis

Mary P. FitzGerald, Dorothea Koch

Loyola University Medical Center, Maywood, IL

Objectives:
Visceral diseases can be characterized by visceral hyperalgesia, referred pain and cutaneous hyperalgesia relevant dermatomes. Our objective was to determine whether subjects with PBS/IC (1) demonstrate hyperalgesia during bladder distension, (2) have discomfort during bladder filling that is referred to dermatomes that differ from controls, (3) have altered cutaneous sensitivity to electrical stimulation in dermatomes to which the bladder refers and (4) demonstrate windup when repetitive cutaneous stimuli are applied to those dermatomes.

Methods:
Subjects were included in the **PBS arm** if they had bladder pain rated >=4/10, urinary frequency >=10/24h, ICSI/ICPI scores >=12 and bladder pain had been present >=3 months. Patients were included in the **stress incontinent (SI) control** arm if they had symptoms of SI, no urge incontinence and had no bladder pain. Patients were included in the **asymptomatic control** arm of the studies if they had no incontinence and no bladder pain. All subjects underwent multichannel cystometry during three bladder fills up to a maximum of 300mL and rated the intensity of any bladder discomfort at every 50mL of bladder fill, using a validated VAS. At 300mL or maximum bladder capacity (MBC), whichever was least, subjects also indicated the body area over which they felt a sensation of bladder fullness/discomfort using the body map. Current perception thresholds to electrical stimulation were tested using the Neurometer (Neurotron Inc, Baltimore,MD) at 2000Hz, 250Hz, 5Hz at C5, T6, T10, T12, S3 dermatomes. Repetitive supra-threshold stimulation in trains of 15 stimuli 2 seconds apart were administered to T12, S3 dermatomes.

Results:
We recruited 8 subjects with PBS, 10 subjects with SUI and 8 asymptomatic controls. PBS subjects demonstrated hyperalgesia to bladder filling, with maximum bladder capacity (MBC) <150mL in 4(50%) of the PBS patients and median pain rating of 7 at MBC. Second and third bladder fills did not cause more pain than the first bladder fill. At MBC, 6 (80%) of PBS subjects indicated they sensed bladder fullness/discomfort at both suprapubic and vulvar sites, a pattern that was present in just one SI subject and no asymptomatic controls. Body maps of bladder sensation did not change with repetitive bladder filling. Current perception thresholds were lowered in PBS subjects in S3 dermatome at 5Hz, but similar to controls at all other sites and stimulating frequencies. No windup of sensation occurred with repetitive cutaneous stimulation, but at some frequencies a significantly more PBS subjects failed to habituate to repetitive stimuli than did controls.

Conclusions:
PBS/IC subjects demonstrate hyperalgesia to bladder filling, may have expanded dermatomes of referral, and may be hypersensitive to electrical stimulation. Lack of habituation to nonnoxious stimuli may be a sign of facilitated pelvic sensory pathways in these subjects.

Epithelially-Derived Factors in Interstitial Cystitis

A. Guyot,[1] A. Evagelidis,[1] C. Gibson,[1] G. da Silva Xavier,[2] G.A. Rutter,[2] J.W. Hanrahan[1] and F. Cervero[3]

Departments of [1]Physiology and [3]Dentistry, McGill University, Montreal, Quebec, Canada and [2]Department of Biochemistry, University of Bristol, Bristol, UK

Urothelial ATP release is upregulated in interstitial cystitis (IC), however the precise role of purinergic signalling in IC and the mechanisms of ATP release are not well understood. To study pain in cystititis we have used gene knockout mice and the cyclophosphamide model. Indices of behaviour and inflammation were assessed after intraperitoneal administration of 100-300 mg/kg cyclophosphamide. Mice lacking $Na_V1.8$, a TTX-resistant sodium channel selectively expressed in peripheral afferent nerves, exhibited pain behaviour similar to that of wild-type mice, both under baseline conditions and after induction of cystititis. By contrast, pain behaviour and inflammation were significantly reduced in mice lacking NK1 or P2X3 receptors. These results implicate substance P and ATP in mediating the pain associated with the cyclophosphamide model, and suggest it may be useful for studying the role of ATP release in cystitis.

To identify possible mechanisms that may contribute to upregulated ATP release in IC at the cellular level, an *in vitro* approach for distinguishing transmembrane from vesicular efflux was developed. In this method firefly luciferase is stably expressed in cells and luminescence is monitored following addition of luciferin to the bath to assay cytoplasmic [ATP]. To improve sensitivity to ATP in the physiological (mM) range, nucleotide affinity of luciferase was reduced by mutagenizing its catalytic site. Calibration of luminescence signals in live HEK cells revealed that resting $[ATP]_i$ was 5.9 ± 0.57 mM under control conditions, and this declined by ~38% during cell exposure to the metabolic inhibitor 2-deoxyglucose, and by >95% after further addition of sodium azide. All cells had similar luciferase protein expression according to immunofluorescence staining with anti-luciferase antibody, and luminescence images obtained with a photon counting camera revealed similar [ATP]. Mechanical stimulation caused a small, but rapid, decrease in $[ATP]_i$ that was not due to cell lysis since $[ATP]_i$ recovered within minutes at 22 ^oC. Interestingly, the release was not affected by pre-incubating cells with BAPTA-AM to lower intracellular calcium, however ATP recovery was Ca^{2+}-dependent. ATP release from the cells under these conditions was confirmed by parallel experiments with extracellular (wild-type) luciferase. We conclude that a large fraction of mechanically-stimulated ATP efflux from HEK cells occurs by a trans-membrane route. Intracellular luciferase method and novel luciferase constructs tethered extracellularly are being used to study ATP release from control and IC urothelial cells.

Complete Characterization of an Antiproliferative Factor from the Urine of Interstitial Cystitis Patients

Susan Keay

University of Maryland and Baltimore VA Medical Center, Baltimore, MD

Approximately one million people in the United States suffer from interstitial cystitis (IC), a chronic painful urinary bladder disorder characterized by thinning or ulceration of the bladder epithelial lining; its etiology is unknown. We previously showed that bladder epithelial cells from IC patients produce an "antiproliferative factor" (APF) whose activity is found in the urine of approximately 95% of IC patients. This APF alters bladder epithelial cell production of specific growth factors (including HB-EGF and EGF), profoundly inhibits bladder cell proliferation, and regulates the expression of at least 13 genes involved in cell adhesion and/or proliferation. Treatment of normal bladder cells with purified APF causes the same changes in growth factor production, gene expression and cell proliferation seen in IC cells, suggesting that this factor may play a critical role in the pathogenesis of IC.

The structure of APF has now been determined and confirmed by total synthesis and measurement of biological activity in both normal bladder epithelial cells and bladder carcinoma cells. APF was determined to be an acidic, heat stable sialoglycopeptide, whose peptide chain has 100% homology to the putative 6th transmembrane domain of frizzled 8. Synthetic APF caused the same changes in cell proliferation and HB-EGF/EGF production seen with purified native APF, confirming its structure. We hypothesize that APF may cause the bladder epithelial abnormalities associated with IC, and propose that it may be useful as a noninvasive diagnostic biomarker for IC.

Mast Cell Migration During PRV-Induced Neurogenic Cystitis

Michael C. Chen and David J. Klumpp

Department of Urology, Feinberg School of Medicine, Northwestern University, Chicago, IL

Interstitial cystitis (IC) is often regarded as a neurogenic cystitis. Several models have been developed to study cystitis *in vivo*, and these have typically involved instillation of noxious stimuli into rodent bladders. While these models are useful for studies of many basic mechanisms, they lack the necessary neural component for modeling neurogenic cystitis. Conversely, feline IC is a highly relevant model, but the lack of genetics and limited access to animals preclude widespread utilization of this model.
To develop a model of neurogenic cystitis that is tractable to genetic analysis, we have adapted the previously characterized system of inducing mast cell-mediated neurogenic cystitis by infection of rats with Bartha's strain of pseudorabies virus (PRV) to infection of mice. PRV infection of mice results in cystitis associated with leukocyte influx and endothelial swelling by day 3 post-infection (PID 3). Staining revealed that increased ICAM-1 expression occurred in $CD31^+$ cells, indicating expression in endothelial cells and providing a basis for leukocyte influx. Vascular permeability was also increased following PRV infection as determined by Evan's blue dye extravasation. Preliminary studies did not identify a significant influx of mast cells (MCs), however, MCs underwent a change in distribution within bladder tissues. Mock-treated animals exhibited a pattern of MC distribution where the majority of MCs resided in the detrusor, and no MCs were observed in the urothelium. In contrast, MCs were most abundant in the lamina propria of bladders from animals infected with PRV, and MCs were even observed in the urothelium. The pool of MCs that migrated to the LP appears specifically derived from the proximal detrusor for distal MC counts were not changed significantly. This shift in mast cell localization was also associated with activation state so that the prevalence of activated mast cells in the lamina propria increased 20-fold in PRV-treated animal relative to controls. Interestingly, PRV infection of TNFR I/II knockout mice failed to induce MC activation and migration. These data suggest that PRV induces differential responses among pools of bladder mast cells that results in activation and migration of proximal detrusor MCs in a model of neurogenic cystitis tractable to genetic analysis.

IL8 Expression in Urothelial Differentiation and Interstitial Cystitis

Monica Liebert and Stephanie Tseng-Rogenski *University of Michigan, Ann Arbor, MI*

Increasing evidence suggestions that interstitial cystitis (IC), a painful bladder syndrome, is associated with a lack or loss of urothelial differentiation, both in patients and a cat model of the syndrome. We have studied the process of urothelial differentiation using an *in vitro* model, and have identified a number of genes associated with urothelial differentiation.

We found, using expression arrays, that IL8 is up-regulated during urothelial differentiation. These data were confirmed by northern and western blotting of RNA and protein extracts, respectively of normal human urothelial cells cultured under undifferentiated and differentiating conditions. Using RT-PCR, we found that bladder biopsies from normal controls showed high levels of IL8, while a significant number of specimens from IC patients failed to demonstrate IL8 signal under identical conditions.

IL8 is a cytokine known to participate in tissue defenses by its chemotactic effect on white cells, especially polymorphonuclear blood leukocytes. Increased IL8 expression in differentiated urothelial cells is consistent with this function in protecting the body from potential urinary bacterial pathogens. However, in other epithelial cells, IL8 has been shown to have additional homeostatic effects. Using siRNA, we were able to significantly inbhibt the level of IL8 protein in differentiated normal human urothelial cells. These cells had reduced adherence to culture substrate compared to controls. Our future studies will continue to evaluate the role of IL8 in urothelial biology.

Furthermore, we will evaluate the expression of IL8 and other cytokines in urine samples using a multiplex proteomic approach. This method will be used to provide simultaneous evaluation of urinary markers of urothelial differentiation in IC patients compared to controls.

Proteomics Approaches to Interstitial Cystitis

Shuzhen Qin, Cindy Williams, Michael P. O'Leary, and Brian C-S Liu

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Our overall goal is to generate disease-associated protein profiles from specimens obtained from patients with interstitial cystitis (IC) and to identify these proteins. The technical challenge to analysis of serum or urinary proteome is that the proteins are present at unequal concentrations. A few are so dominant, such as albumin and immunoglobulins, that they mask detection of other proteins. Because of these high abundance proteins, current technologies, while theoretically capable of analyzing protein amounts spanning four orders of magnitude, are only able to analyze proteins ranging over two orders of magnitude and cannot analyze the lower abundance proteins that may be the next biomarkers and drug targets. To facilitate the identification of low abundance proteins, we fractionated serum and/or urine samples from patients with interstitial cystitis and from normal controls using multi-dimensional liquid chromatography, which separates proteins into distinct pH ranges and hydrophobicity. Differential expression of proteins from fractions was then determined and identified by two-dimensional difference gel electrophoresis (2-D DIGE) and tandem mass spectrometry (ms/ms)-based sequence identification. Results demonstrate improved resolution of proteins on 2-D DIGE following multi-dimensional liquid chromatography when compared to the unfractionated samples. Several proteins that were differentially expressed in urine specimens from patients with IC were identified to be involved with inflammation. These proteins include various cell surface adhesion molecules, bradykinin, prostaglandin D2, and beta-2-glycoprotein I. Although speculative, of interest is that beta-2-glycoprotein I may bind to endothelial cell surface and leads to endothelial cell activation, which is manifested by upregulation of cell surface adhesion molecules and increased secretion of IL-6 and prostaglandins. The expression of these proteins may contribute to the clinical symptoms found in IC. Our results demonstrate that the use of proteomics approaches to IC, coupled with reducing the complexity of the serum and urine proteome, may facilitate the identification of low abundance proteins and add to our ability to find biomarkers that may be useful for drug discovery and/or diagnostic tools.

Urinary Uronate and Sulfated Glyacosaminoglycan Levels: Markers for Interstitial Cystitis Severity

Vinata B. Lokeshwar,[1] Marie G. Selzer,[1] Wolfgang H. Cerwinka,[1] Maria-Fernanda Lorenzo Gomez,[1] Robert R. Kester,[2] Darwich E. Bejany,[3] Angelo E. Gousse[1]

[1]Departments of Urology, University of Miami, School of Medicine, Miami, FL, [2]Urology Practice, Lewiston, MA, and [3]Cedars Medical Center, Miami, FL

Introduction:
Urologists frequently rely on symptom and problem indices to monitor interstitial cystitis (IC) patients. Uronic acid is a component of most glycosaminoglycans (GAGs), a protective coating to the bladder urothelium. We evaluated whether urinary uronate and sulfated GAG levels correlate with IC severity and characterized urinary GAG species.

Methods:
Thirty-seven IC patients from 3 community offices and 1 academic center and 14 normal volunteers were included in the study. All patients fulfilled the NIDDK criteria, except for glomerulation. All study individuals completed the O'Leary and Sant problem and symptom questionnaire. Group I patients: One/both indices < 50% maximum score; Group II: Both indices ≥ 50% maximum score. Urinary uronate was fractionated using cetyltrimethylammonium bromide (CETAB). Uronate and sulfated GAG levels in urine, CETAB-precipitates and -supernatants were measured by Bitter and Muir and Farndale assays, respectively and normalized to creatinine (μg/mg Cr). GAG species were analyzed by agarose gel electrophoresis.

Results:
Mean urinary uronate levels were elevated in Group II (1614 ± 904.6) when compared to normals (612.4 ± 327.2) and Group I (593.8 ± 422.1) ($P < 0.001$) regardless of glomerulation and treatment. A small portion of urinary uronate was CETAB-precipitable, representing macromolecular GAGs. Uronate levels in CETAB-precipitate were 2.8-fold elevated (8.0 ± 5.07) in Group II patients when compared to normals (2.89 ± 1.6) and Group I (3.35 ± 1.936) ($P < 0.001$). Uronate levels in CETAB supernatants were elevated 2.7-fold in Group II patients (1393 ± 671.9) when compared to Group I (509.3 ± 331.3) and normals (503.3 ± 316.3). Two distinct types of GAG species, slow- and fast moving, were present in Group II patients. The slow-moving GAG partitioned in CETAB precipitates, whereas, the fast moving species remained in CETAB supernatants. Sulfated GAG species in Group II IC patients were 2.0-fold elevated (36.57 ± 14.3) when compared to normals and Group I patients ($P < 0.001$). At 1100 μg/mg Cr cut-off limit uronate levels showed high accuracy (86.2%), specificity (92.3%) and sensitivity (80%). Patients with uronate levels ≥ 1100 μg/mg Cr had 11.2-fold increased risk of having severe disease (i.e., both indices ≥ 50% or Group II patients). For sulfated GAGs, the cut-off limit of 20 μg/mg Cr showed high sensitivity (88%), and reasonable accuracy (78.4%) and specificity (69.2%).

Conclusion:
The majority of urinary GAGs likely exist as small oligosaccharides. Urinary uronate and sulfated GAG levels are elevated in IC patients with severe disease and may become useful and inexpensive markers in monitoring IC severity.

Development of Urinary Bladder Autoimmune Cystitis Model (Progress Report)

Yi Luo

The University of Iowa College of Medicine, Iowa City, IA

Introduction and Objective:
Interstitial cystitis (IC) is a chronic irritable condition of the urinary bladder with an undefined etiology at the present time. However, there is evidence suggesting that an antigen-induced immune/autoimmune inflammatory process is involved in the pathogenesis of this disease. Our objectives are to create a transgenic (Tg) autoimmune cystitis model and determine the role of T helper (Th) polarization in the development of bladder autoimmune disease in this model.

Methods:
A plasmid DNA (pUPII-OVA) containing the urothelium-specific uroplakin II gene promoter and a chimeric transgene consisting of human transferrin receptor transmembrane domain and chicken ovalbumin (OVA) was constructed by DNA cloning. The 6.1 Kb KpnI-DraIII fragment of pUPII-OVA was microinjected for the development of proposed Tg mice (termed as URO-OVA Tg mice). Tail genotyping was performed for Tg screening and Tg founders were backcrossed with C57BL/6 mice. Various tissues were prepared from F1 mice and processed for RT-PCR and ELISA. The effectiveness of $CD4^+$ T cells as effectors in the induction of OVA-mediated cystitis was tested using OVA CD4 T cell receptor (TCR) Tg mice (OT-II mice).

Results:
The constructed plasmid pUPII-OVA (~8.9 Kb) resulted in a cell-type specific OVA expression in transient expression transfection assays. Among the 82 pups produced, 6 Tg founders were obtained. Four Tg founders produced a total of 23 F1 offspring with 16 pups being positive by PCR tail DNA screening (One founder did not produce positive offspring). To determine the specificity of OVA expression, 1 mouse (F1 generation) from each 3 positive lines was processed for tissue RNA extraction and RT-PCR analysis. Among various tissues tested, the bladder was found to be the predominant organ for OVA expression while skin also showed weak OVA expression. Densitometry analysis indicated that OVA mRNA expression was 4-20 fold higher in the bladder than in the skin. To further evaluate OVA expression, the bladders and skins were processed for protein extraction and ELISA analysis. Interestingly, although two Tg lines tested showed comparable expression of OVA in the bladders, one Tg line showed no detectable OVA expression in skin whereas the other line showed clear OVA expression in skin. Currently, these bladder positive lines are being crossbred with C57BL/6 mice for the production of F2 Tg generation.

Naïve OT-II Tg mice developed bladder inflammation after 5 doses of intravesical OVA administration (10 mg/dose; once a week). OT-II $CD4^+$ T cells also demonstrated to be effective on induction of cystitis after adoptive transfer (1 X 10^7 cells) into wild-type C57BL/6 mice followed by 1 dose of intravesical OVA administration (10 mg).

Conclusions and Future Directions:
Three URO-OVA Tg lines have been developed. They all exhibit high OVA expression in the bladder and low OVA expression in skin. Further characterization has currently being undertaken. OT-II $CD4^+$ T cells have demonstrated to be potent effectors in the development of OVA-mediated cystitis. They will be differentiated into Th1 and Th2 cells and used to determine the role of Th polarization in the development of autoimmune cystitis.

Tryptase Activation of Calcium-Independent Phospholipase A_2 in Human Bladder Endothelial Cells

Jane McHowat, Alice Rickard, Suzanne Vinson

Saint Louis University School of Medicine, St. Louis, MO

Increased mast cell numbers and mast cell activation is one of the prevalent etiologic theories for interstitial cystitis (IC). We hypothesize that activation of mast cells can initiate a cycle of inflammation in the bladder wall, with tryptase released from the mast cells activating protease-activated receptors (PAR) on either urothelial cells of the bladder wall and on endothelial cells lining the vasculature, resulting in increased Ca^{2+}-independent phospholipase A_2 ($iPLA_2$) activity and increased production of membrane phospholipid-derived inflammatory mediators such as eicosanoids and platelet-activating factor (PAF). This study tests this hypothesis in bladder endothelial cells and examines the effects of tryptase on endothelial cell phospholipase A_2 (PLA_2) activity and the resultant increase in inflammatory phospholipid metabolite accumulation that may propagate the inflammatory process. We stimulated human bladder microvascular endothelial cells (HBMEC) with tryptase and measured PLA_2 activation and the production of multiple membrane phospholipid-derived inflammatory mediators. Tryptase stimulation of HBMEC resulted in activation of a calcium-independent phospholipase A_2 ($iPLA_2$), leading to an increase in arachidonic acid and prostacyclin (PGI_2) release and increased production of platelet activating factor (PAF). These responses were all blocked completely by pretreatment of HBMEC with the $iPLA_2$-selective inhibitor bromoenol lactone (BEL). The combination of increased PGI_2 and PAF production in the bladder circulation may result in vasodilation and increased polymorphonuclear leukocytes (PMN) adherence to the endothelium and may facilitate recruitment of PMNs to the bladder wall of IC patients. Endothelial cell PAF production contributes importantly to the recruitment of leukocytes and monocytes to inflamed tissue by promoting adhesion to the endothelium and thus can play a major role in the progression of inflammatory diseases such as IC. In addition to increased PAF expression on the endothelial cell surface, increased cell surface expression of P-selectin contributes to the early stages of cell adherence to an activated endothelial cell layer. The combination of increased PAF and P-selectin expression on the HBMEC surface would suggest increased recruitment of inflammatory cells. Tryptase stimulation of HBMEC resulted in significant increases in P-selectin expression on the cell surface that was completely inhibited by BEL pretreatment. We pretreated HBMEC with methyl arachidonyl fluorophosphonate, an inhibitor that was originally designed as a cytosolic PLA_2 inhibitor, but has subsequently been shown to inhibit PAF acetylhydrolase. MAFP-pretreated HBMEC demonstrated a significant increase in PAF production and P-selectin cell surface expression in response to tryptase stimulation. In turn, this resulted in significant increases in PMN adherence to HBMEC monolayers. Taken together, these data suggest that release of tryptase from activated mast cells may directly contribute to the propagation of inflammation in IC by recruiting inflammatory cells to the bladder wall.

Neurogenic Pathogenesis of Interstitial Cystitis

Michael A. Pezzone

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Chronic pelvic pain is a poorly understood but sufficiently debilitating clinical condition primarily affecting women. Few diagnostic and treatment options are available for this understudied patient population which is estimated at 9.2 million in the United States. The causes of chronic pelvic pain are numerous but may involve gynecologic, urologic, gastrointestinal, musculoskeletal, neuronal, or psychological origins as well as combinations thereof. The urinary bladder and colorectum are two of the larger pelvic organs thought to be affected primarily in these disorders, and thus, it is not surprising that interstitial cystitis (IC) and irritable bowel syndrome (IBS) are two of the commonest causes of chronic pelvic pain. The observed overlap of chronic pelvic pain disorders such as IC and IBS suggests a common underlying etiology or even cross-organ (neurogenic) sensitization.

Using a newly developed rodent model for studying afferent-mediated interactions of the pelvic organs in the rat, we will investigate the hypothesis that chronic irritation of the distal colon may adversely influence and sensitize urinary bladder afferents leading to neurogenic cystitis and its associated physiologic sequelae. The studies proposed in this application will attempt to shed more light on the overlap and etiology of chronic pelvic pain syndromes and the role of cross-organ, afferent sensitization.

The Role of the NF-κB Signaling Pathway in the Pathogenesis of Interstitial Cystitis

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Information transfer from the cellular membrane to the nucleus involves several well-characterized molecular signaling pathways that lead to transcriptional activation. One of the most important pathways of information transfer is the NF-κB (nuclear factor-κB) signaling pathway involved in the regulation of an exceptionally large number of genes responsible for a cellular response in an organ system such as the bladder to inflammation, infection, and other stressful cellular situations that requires rapid reprogramming of gene expression. While the exact etiology of interstitial cystitis (IC), a debilitating, chronic inflammatory syndrome of the bladder remains elusive, the common translational factor between proposed clinical etiologies is that they are all known to be cellular activators of the intracellular NF-κB signaling pathway. **We hypothesize that the NF-κB signaling pathway is the essential inflammatory, immunoregulatory, and neuromodulatory mechanism for the pathogenesis of the bladder response in IC.** We are testing this hypothesis by characterizing the NF-κB signaling pathway and regulated cellular genetic expression in bladder tissue as well as urothelial cell cultures from IC patients compared to normal controls. In addition to establishing NF-κB as an essential signaling pathway for the bladder response in IC, these aims will determine whether the activated signaling represents a dysfunctional (aberrant internal cellular control of the signaling pathway) versus functional (signaling pathway response to external cellular stimulation) urothelial response to extracellular stimulation. The importance of determining the signaling response has relevance to the development of diagnostic gene (dysfunctional inhibitor or kinase proteins due to gene mutations) or post-transcription protein markers that give rise to aberrant NF-κB regulation. Furthermore, treatments based on the pathogenesis of IC would focus on modulatation of the extracellular stimulation and downregulation of acute and chronic hyperactivation in the functional response versus developing interventions that address the molecular defects associated with the dysfunctional response.

Effects of Early-In-Life Bladder Stimulation on Adults

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Inflammation in neonates has developmental effects on somatic and visceral nociceptive systems. The present study examined the effects of inflaming the bladders of either neonatal (2 week old) or adolescent (4 week old) rats on several response measures as adults (12-15 weeks old). The adult response measures included micturition frequency, viscero-motor (abdominal EMG) and arterial blood pressure (ABP) responses to urinary bladder distention (UBD), and mechanical and thermal somatic response thresholds. Neonatal and adolescent groups of female Sprague Dawley rats received intravesical administration of either zymosan (1%) or saline via a urethral catheter, or anesthesia alone (2% halothane). Neonatal and adolescent rats received 3 and 14 consecutive days of treatment, respectively, where each daily treatment was 30 min in duration. Rats were then tested in a blinded fashion as adults. Baseline micturition frequency and baseline response thresholds to thermal and mechanical stimuli applied to the hindpaws were measured first. Then, all of the early-in-life pre-treatment groups were rechallenged with either 1% zymosan or saline under brief halothane anesthesia for a 30 min period. Micturition frequency (12 h post zymosan or saline) and somatic response thresholds (1 and 24 h post zymosan or saline) were reassessed. ABP and EMG responses to phasic, graded (10-80 mmHg, 20 s) UBDs were determined under light halothane anesthesia one day after zymosan or saline. Neonatal and adolescent pretreatment of the bladder with zymosan resulted in a significant increase in baseline micturition frequency relative to either saline or anesthesia alone pretreatments. Interestingly, the increased spontaneous micturition frequency in rats pretreated with zymosan decreased following subsequent administration of zymosan as adults. Neonatal, but not adolescent rats, pretreated with zymosan showed significantly greater EMG responses to UBD when rechallenged with zymosan as adults as compared to anesthesia alone pretreated rats. ABP significantly increased with UBD pressure, but there were no significant differences in ABP responses to UBD across treatment groups. None of the early-in-life bladder treatments differentially affected either mechanical or thermal baseline response thresholds as adults. Similarly, early-in-life treatments did not differentially affect somatic response thresholds following adult reexposure to either zyomsan or saline. These findings suggest that inflammation of the bladder in neonates can produce hyperalgesia to UBD as adults. Moreover, inflammation of the bladder in either neonates or adolescents increases baseline micturition frequency as adults. These animal data suggest that exposure to early-in-life inflammatory (painful) events may in part predispose the organism to later-in-life susceptibility to two characteristics of interstitial cystitis, pain and increased frequency of micturition.

PACAP Overexpression and Voiding Function

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Ronald W. Wood, Edward M. Messing,
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There are numerous complementary and competing hypotheses regarding the etiology and pathogenesis of interstitial cystitis (IC). We intend to test the hypothesis that symptoms develop when a barrier defect results in enhanced expression of neuropeptides that effect signal transmission nervous system. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a leading candidate neuropeptide in the regulation of bladder function, and upregulates in bladder afferent cells in the dorsal root ganglia (DRG) in cystitis models. It has been demonstrated recently that PACAP antagonists delivered intrathecally or intravesically can improve bladder overactivity in rodents with cystitis. We are developing two transgenic mouse models of tissue specific PACAP over-expression to evaluate the role of this factor in the neurotrophic mechanisms and lower urinary tract plasticity associated with cystitis. These mouse models will address not only PACAP's contribution to micturition, but also it's role in nociceptive mechanisms in general. In a somatic mosaic model, PACAP38 will be over-expressed selectively in bladder sensory neurons following a direct bladder wall injection of a recombinant retrovirus that expresses the cDNA from the neuronal enolase specific promoter. We will also make mice that harbor a PACAP38 transgene whose transcription is controlled by a tetracycline-inducible promoter. These mice (TREPACAP38) will be crossed with our UPII-rtTA transgenic mice that express the reverse tetracycline transactivator only in urothelial cells. These bitransgenic mice (Uro-TetON-PACAP38) will receive doxycycline in their drinking water to evaluate the effect of drug-inducible PACAP over-expression on bladder function. To evaluate bladder function mice are placed in metabolism cages over electronic balances. Computerized sampling of mass at 10 times per second is used to detect urine voiding events. A photocell monitors locomotor activity. Void volume, frequency, urine production rate, void duration, peak and sustained uroflow rate, hematuria, body temperature and weight, rate of beam interruption, frequency of pauses, and proportion of session spent pausing are routinely measured. Urothelial permeability and sensitivity are assessed with sodium fluorescein and potassium chloride respectively. Somatic mosaic and inducible PACAP expression are expected to significantly alter bladder function.

Mouse Bladder Transcriptome in Response to BCG Therapy

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Introduction:
Bacillus Calmette-Guerin (BCG) has been successfully applied to the treatment of superficial bladder cancer for over two decades and has been presented as a promising option for treatment of interstitial cystitis. However, the mechanisms underlying BCG therapy are not completely understood. In addition, intravesical BCG therapy induces bladder inflammation.

Objectives:
We sought to determine bladder genes that are sensitive to BCG therapy apart from those whose expression is altered in response to overall inflammation.

Methods:
For this purpose, female mice were instilled with one of the following treatments: saline, BCG, TNF-alpha, or LPS. Following intravesical instillation for 1, 2, and 4 weeks, mouse bladders were removed and placed on RNAlatter. The urothelium and submucosa were separated from the detrusor muscle and the RNA was extract for cDNA analysis using Clontech 5K mouse atlas array.

Results:
Array results were normalized and genes significantly expressed ($p<0.001$) were selected for further analysis. Those genes were sorted by the ratio between treatment and control and only genes with a ratio > 3.0 were selected for analysis. Next, we sorted the genes that were up-regulated in response to BCG but not to LPS or TNF-alpha. Finally, genes that fulfill all the requirements listed above were considered BCG-responders (n=146) and submitted to promoter analysis and interaction network generation tool for gene regulatory network identification (PAINT)[3]. The set of BCG-responders were compared to the reference set containing all 5,000 genes present on the clontech array. In addition, 2 different cluster analysis (SOMs) were performed using all significantly expressed genes ($p<0.001$). The first SOM was performed using the expression values and the second using the ratios. Transcription factors that were significantly ($p<0.01$) and specifically altered by BCG included: AP-1, HNF, USF, and Pax-4.

Conclusion:
In addition to NF-κB and AP-1 known to be activated by BCG, promoter analysis in combination with cDNA array technology provides a substantially pruned list of genes and transcription factors that can be examined in detail in further experimental studies on BCG-induced gene regulation.

Urothelial Barrier Function and Interstitial Cystitis

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Chronic inflammatory uropathies, which subsume interstitial cystitis, encompass a spectrum of syndromes characterized by pain and frequency of voiding, inflammation and damage to the urothelium. The processes underlying the genesis and progression of these conditions is poorly understood. Our aim is to test the hypothesis that a failure of the urothelium to fulfil an effective urinary barrier function is an initiating factor in at least a subset of disease. The hypothesis predicts that urothelial cells in some IC patients do not achieve fully functional cytodifferentiation, and we are testing the hypothesis by a) examining expression of urothelial differentiation-associated antigens and b) by in vitro assay of urothelial cytodifferentiation and barrier function. The work will be important in establishing whether urothelial cells from patients with IC are inherently abnormal, or whether the aetiopathology of IC is secondary to other factors.

The urothelium is highly specialised to function as a urinary barrier, with a transcellular barrier provided by the thickened plaques of asymmetric unit membrane decorating the apical surface of the terminally differentiated superficial cell and a paracellular barrier provided by intercellular tight junctions. These characteristics are acquired as a function of the urothelial cytodifferentiation program and we have developed cell culture systems to allow human urothelial cells to be isolated, propagated as monocultures and tested for their ability to differentiate in two differentiation models.

a) Pharmacological induction of the urothelial differentiation program by means of antagonism of the epidermal growth factor-dependent autocrine loop and activation of peroxisome proliferator activated receptor gamma.

b) Development of a biomimetic urothelium for testing of physiological function in terms of transepithelial resistance.

Preliminary data from an immunohistochemical assessment of urothelial differentiation in archived specimens of histologically-proven IC suggests that, even in the presence of identifiable superficial cells, there are apparent aberrations in expression of urothelial differentiation-associated antigens. Secondly, we have adapted our urothelial cell culture techniques for small cystoscopic biopsies from patients with a range of dysfunctional bladders, with overactive bladders with detrusor muscle instability included as negative controls. Urothelial cell cultures have been successfully initiated using this approach and preliminary results of the differentiation and barrier function potential of IC-derived urothelium will be presented.

Regulation of Urothelial Growth and Differentiation

Tung-Tien Sun, Andrew Hu, Xiangtian Kong, Fangming Deng, Fengxia Liang, Ge Zhou, Liyu Tu and Gert Kreibich

Epithelial Biology Unit, Departments of Dermatology, Pharmacology, Urology and Cell Biology, New York University Medical School, New York, NY

Uroplakins are a group of integral membrane proteins made by terminally differentiated urothelial cells as their major specialization products. They form 16 nm particles packed hexagonally to form 2D crystals (urothelial plaques) covering almost the entire apical urothelial surface. It has been reported that urothelial expression of uroplakins is abnormal in interstitial cystitis. To better understand how the four major uroplakin proteins assembly into the plaque, we transfected COS-1 cells using single or pairs of uroplakin cDNAs. This experiment demonstrated that the tetraspanin uroplakins Ia and Ib interact with UPII and UPIII, respectively, to form UPIa/II and UPIb/III heterodimers which can then exit from the ER. The prosequence of proUPII then undergoes secondary modifications including additional glycosylation leading to the formation of complex sugars and proteolytic cleavage by furin. Using a panel of novel conformation-dependent monoclonal and polyclonal antibodies to uroplakins, we showed that secondary modifications play a key role in the assembly of urothelial plaques and may play a role in suppressing plaque assembly in cultured urothelial cells. In another series of experiments we demonstrate that genetic ablation of uroplakins II and III genes leads to the loss of 16 nm particles and urothelial plaques, compromised barrier function, absence of umbrella cells, vesicoureteral reflux and, when combined with a certain genetic background, leads to renal dysfunction and postnatal death. These results suggest that uroplakins are integral subunits of urothelial plaques and contribute to the permeability barrier function; that the insertion of uroplakin plaques into the apical surface contributes to the expansion of apical surface area and the formation of large umbrella cells; and that urothelial abnormalities can contribute to a number of important urinary tract problems including vesicoureteral reflux, ureteral obstruction, hydronephrosis and possibly renal failure and death.

Neurotrophic Mechanisms in LUT Plasticity With Cystitis

Margaret A. Vizzard

University of Vermont College of Medicine, Burlington, VT

Interstitial cystitis (IC) is a chronic inflammatory bladder disease syndrome characterized by urinary frequency, urgency, suprapubic and pelvic pain. We hypothesize that pain associated with IC involves an alteration of visceral sensation/bladder sensory physiology. Altered visceral sensations from the urinary bladder that accompany IC may be mediated by numerous factors. The central hypothesis of our recent work is that the vasoactive intestinal polypeptide (VIP)/pituitary adenylate cyclase activating polypeptide (PACAP) system is a prominent modulator of bladder sensation and function and that inflammation-induced changes in neurotrophic factors and/or neural activity arising in the bladder alter PACAP/PACAP receptor expression in the lower urinary tract (LUT) to mediate altered micturition function in IC.

1. We have examined changes (neurochemical, organizational) in micturition reflexes using cyclophosphamide (CYP)-induced cystitis. We have demonstrated increases in the expression of substance P, calcitonin gene-related peptide and PACAP in bladder afferent cells in the lumbosacral dorsal root ganglia and spinal cord. We have identified PAC1 receptor isoforms in LUT tissues and have demonstrated that intrathecal or intravesical administration of PACAP antagonist (10 or 50µg) reverses bladder overactivity induced by cystitis. VIP and PACAP have well-established direct contractile effects on a variety of smooth muscles in a species- and tissue-specific manner. Our recent studies on isolated bladder strips demonstrate that 50-100 nM PACAP elicits a transient contraction and a sustained increase in the amplitude of spontaneous phasic contractions and also increases the amplitude of nerve-mediated contractions. Incubation of bladder strips in 1 µM tetrodotoxin prior to PACAP application did not alter observed increases in spontaneous phasic contractions, indicating that PACAP does not mediate its effects by facilitating presynaptic neurotransmitter release but elicits a direct contractile response from bladder smooth muscle.

2. Possible mechanisms underlying the neural plasticity after CYP-induced cystitis may involve alterations in neurotrophic factors and/or the production of pro-inflammatory cytokines in the urinary bladder. Recent studies from this laboratory have demonstrated changes in the mRNA/protein expression of a number of neurotrophic factors in the urinary bladder and pelvic ganglia. We determined NGF dependence of CYP-induced changes in bladder function by using a recombinant NGF sequestering protein (REN1820). Rats examined 4 hr or 48 hr after CYP treatment + REN1820 exhibited significantly ($p \leq 0.01$) fewer non-voiding contractions (NVCs) with smaller amplitude. Rats examined 48 hr after CYP treatment + REN1820 exhibited decreased ($p \leq 0.01$) voiding frequency. No changes in filling, threshold or micturition pressures were observed with REN1820 treatment. Rats treated with CYP + REN1820 exhibited greater mobility and normal resting postures compared to rats treated with CYP + vehicle.

These studies suggest that dramatic alterations in micturition reflex organization and function occur after cystitis and may be mediated, in part, by production of neurotrophic factors in the urinary bladder.

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Two Studies:
1. Events Preceding Interstitial Cystitis (EPIC)
2. Maryland Genetics of Interstitial Cystitis (MaGIC)

J. W. Warren, S. Ashley, V. Brown, A. Hopkins, L. Horne, A. Jewell, S. Keay, P. Langenberg, D. Meyers, K. Tracy, J. Xu, J. Zhu

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This group is performing two NIDDK-sponsored basic research studies in interstitial cystitis (IC). Each will use common recruitment strategies. Data are collected to determine whether cases can be defined by NIDDK, O'Leary-Sant, University of Wisconsin, Parson's PUF, or the International Continence Society's Painful Bladder Syndrome criteria. We believe that appropriate data are collected to additionally incorporate an emerging new definition of IC.

1. Events Preceding Interstitial Cystitis (EPIC)

This is a case control study of IC with four aims:
1) To seek risk factors for IC
2) To seek associations of non-bladder syndromes with IC
3) To determine whether urine APF, HBEGF, and/or EGF are associated with IC early in the disease process
4) To determine the natural history of IC

This is a national study conducted by telephone, mail and email. To avoid confounding by prostititis, we are studying only female cases. Through urologists, the ICA and the Internet, we are recruiting incident cases, i.e. with symptoms of IC beginning no more than 12 months earlier. Control women will be matched by age and national region and will be recruited by random digit dialing. Recruitment began in mid-March, 2004 and to date we have screened 279 potential participants and interviewed 81 incident cases. Control recruitment will start after the first batch of 100 cases. We are seeking 400 cases and 400 controls.

2. Maryland Genetics of Interstitial Cystitis (MaGIC)

This group, with the help of the ICA and the Fishbein Family Foundation, earlier determined that the prevalence of IC in first degree relatives of IC patients was 17 times what one would expect in the general population and that the concordance of IC in identical twins is much greater than in fraternal twins. Both findings are consistent with a genetic susceptibility to IC in some families.

This project has two specific aims:
1) To develop a registry of multiplex families (i.e. with two or more first degree relatives with IC)
2) Using linkage analysis within these families, to seek alleles of susceptibility to IC

Each member of a multiplex family is assigned an intermediate definition of IC (Definite, Probable… Not IC). Urine for biomarker testing and blood for DNA extraction are requested from all participating family members. Genome-wide linkage analysis will be performed by the Center for Inherited Diseases Research at Johns Hopkins and analysis will be conducted by Drs. Meyers and Zhu at Wake Forest University.

We received permission to begin this NIDDK Center grant in June, 2004. We have 106 multiplex families who have fulfilled preliminary screening for participation. Additionally, before we begin active recruitment, 176 other patients have contacted us inquiring about participation. We are seeking 400 multiplex families and will begin additional active recruitment in the spring 2005.

Two Studies:
1. Events Preceding Interstitial Cystitis (EPIC)
2. Maryland Genetics of Interstitial Cystitis (MaGIC)

Continued from previous page.

CONTACT US FOR ELIGIBLE PARTICIPANTS:

EPIC study:
Women with IC symptoms for 12 months or less

OR

MaGIC study:
IC patients claiming one or more first-degree relatives (parent, sibling, child) also with IC

1-877-STUDY IC

studyic@medicine.umaryland.edu

Pain and Interstitial Cystitis

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Pain is a prominent characteristic of interstitial cystitis (IC). Data from the ICDB Study (Simon 1997) show that 93.6 % of the patients enrolled reported having some pain on some part of their body. IC shares many characteristics with other chronic visceral pain syndromes. In addition, many patients with IC report extra-bladder pain symptoms, such as gastrointestinal, pelvic and chronic somatic pain. Thus, women with IC may present with a complex clinical picture of several different pain syndromes in different body areas. These observations suggest that there might be generalized alterations in pain modulatory mechanisms in IC. Although IC has been traditionally viewed as a bladder disease, there is increasing clinical and epidemiological evidence of non-bladder related symptoms and co-occurrence of IC with other chronic pain syndromes, raising the question of systemic rather than local mechanisms.

We suggest a new concept to approach chronic pain in IC, based on 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.

Supported by NIH grants DK57315 (NIDDK) and DK066641 (NIDDK)

Bladder Pain Gene Therapy With Opioid cDNA

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Michael B. Chancellor,
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Interstitial cystitis is a bladder hypersensitivity disease associated with bladder pain, which has been a major challenge to understand and treat. We hypothesize that targeted and localized expression of endogenous opioid peptides in the bladder could be useful for the treatment of bladder pain. Pro-opiomelanocortin (POMC) and preproenkephalin (PPE) are such precursor molecules for endorphins and enkephalins, respectively. In this study, we developed a gene-gun method for the transfer of POMC or PPE cDNA in vivo, and investigated the therapeutic effect on bladder nociceptive responses induced by bladder irritation in rats.

1. Gene Therapy of Bladder Pain with Gene Gun Particle Encoding Pro-opiomelanocortin (POMC) cDNA

Materials and Methods:
Human POMC cDNA was cloned into a modified pCMV plasmid and delivered into the bladder wall of adult female rats by direct injection or gene-gun. Three days after gene therapy, continuous cystometrograms (CMGs) were performed under urethane anesthesia by filling the bladder (0.08 ml/min) with saline, followed by 0.3% acetic acid. Bladder immunohistochemical testing was used to detect endorphin after POMC cDNA transfer.

Results:
Intercontraction interval (ICI) was decreased after intravesical instillation of acetic acid (73.1% or 68.1% decrease) in two control groups treated with saline or gene gun without POMC cDNA, respectively. However, rats which received POMC cDNA via gene-gun showed a significantly reduced response (ICI 35.0% decrease) to acetic acid instillation whereas this antinocicepive effect was not detected in the group of plasmid POMC cDNA direct injection. This effect induced by POMC gene gun treatment was reversed by intramuscular naloxone, an opioid antagonist (1mg/kg). Increased endorphin immunoreactivity with anti-endorphin antibodies was observed in the bladder from gene gun treated animals.

2. Gene Gun Particle Encoding Preproenkephalin (PPE) cDNA Produces Analgesia Against Capsaicin Induced Bladder Pain in Rats

Materials and Methods:
PPE plasmid cDNA was cloned into a modified pCMV plasmid and delivered into the bladder wall of adult female rats by direct injection or gene-gun. Four to 7 days after gene therapy, continuous CMG was performed under urethane anesthesia by filling the bladder (0.04 ml/min) with saline, followed by 15 μM capsaicin. Bladder immunohistochemical testing was used to detect enkephalin after PPE cDNA transfer.

Bladder Pain Gene Therapy With Opioid cDNA

Continued from previous page.

Results:
ICI was decreased after intravesical instillation of capsaicin (65.0% and 63.1% decrease) in the control group or direct PPE gene injection group, respectively. However, in the gene-gun treated group showed a significantly reduced response to capsaicin instillation at day 4 and day 7 (ICI 16.2% and 42.8% decrease, respectively). This analgesic effect was reversed by intravenous naloxone, an opioid antagonist (5mg/kg). Increased enkephalin immunoreactivity in bladder was observed from gene gun treated group at day 4, which was reduced at day 7.

Overall, using gene-gun methods, POMC or PPE gene can be transferred in the bladder and that subsequent increases in expression of endorphin or enkephalin, respectively, in the bladder can suppress nociceptive responses induced by bladder irritation. Thus, these results could support potential clinical application of POMC or PPE gene-gun delivery system for the treatment of bladder pain and other types of visceral pain.

Supported by NIH R21 DK066095

Non-Invasive Assessment of Vascular Response to Acute Cystitis

J.D. Woods, G.P. Lahvish, Z-Y Wang,
J.P. Weichert, D.E. Bjorling

POSTER 1

The purpose of this study was to evaluate the capacity of non-invasive imaging of the bladder (micro-computed tomography or micro-CT) to detect the presence of cystitis using plane and contrast studies. The availability of a unique proprietary contrast agent allowed us to identify that vasculature on micro-CT images, and micro-CT images were compared to histological images obtained from mice sacrificed immediately after scanning.

Cystitis was induced by intraperitoneal injection of cyclophosphamide (CYP; 300 mg/kg). Control mice received an intraperitoneal injection of a similar volume of normal (0.7%) saline. To reduce the mineral content of urine and feces, mice were placed on a liquid diet 72 hours prior to scanning. The diet was switched to a 10% sucrose solution 24 hours prior to scanning. Forty-eight hours after IP injection of CYP or saline, anesthesia was induced with pentobarbital (30 mg/kg, IP), and plane and contrast images were obtained using a micro-CT scanner (ImTek). This device has a resolution of 40 microns. Subsequent to CT imaging, mice received an intraperitoneal injection of the anticoagulant refludan (1 ul/gm at 2 mg/ml). Ten minutes after injection, the thorax was opened and a cannula was inserted into the left ventricle. Mice were perfusion with PBS (60 ml), followed by 6% FITC conjugated *L. esculentum* lectin (Vector Laboratories, Inc.) in 1% BSA-PBS (30 mls). Plain PBS (30 mls) was then infused, and tissues were fixed by perfusion of 10% neutral buffered formalin. Gelatin (150 ml) was injected into the lumen of bladders immediately prior to fixation to help maintain bladder shape during embedding and sectioning. After fixation, bladders were excised and embedded in a gelatin matrix. Transverse serial sections were obtained perpendicular to the bladder axis at 100 mm intervals using a Leica vibratome. Sections were mounted on slides and imaged using a Zeiss Axioplan fluorescent imaging system.

Thickening of the bladder wall could be detected on plane micro-CT images of mice injected with CYP relative to saline-treated mice. Contrast micro-CT images demonstrated increased vascularity of the bladder wall that correlated positively with histological images. Image reconstruction is ongoing, but it appears that micro-CT may be an effective, noninvasive technique for evaluation of bladder contour, thickness, and vascularity in patients with bladder irritation and inflammation such as interstitial cystitis (IC).

Proteomic Characterization of Interstitial Cystitis Bladder: Searching for New Markers

Fernando de Miguel, Suzy van Le, Yukio Hayashi, Naoki Yoshimura, Michael B. Chancellor

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POSTER 2

Please see "Speaker Abstracts" section.

Bladder Pain Gene Therapy With Opioid cDNA

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POSTER 3

Please see "Speaker Abstracts" section.

Urothelial Barrier Function in Interstitial Cystitis

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POSTER 4

The precise aetiology of interstitial cystitis (IC) remains unclear but is likely to be multifactorial. Further understanding of the pathophysiology of IC is essential to the development of future treatment strategies. One of the primary functions of urothelium is to act as an effective barrier, preventing the urine from causing damage to the underlying tissues. The aim of our study is to investigate the postulated aetiology of an ineffective or 'leaky' urothelium as the major contributing factor in the development and progression of IC.

We have obtained multiple small biopsy samples from patients undergoing cystoscopic examination for IC and other non-inflammatory conditions of the bladder, for example overactive bladder. One sample was fixed for immunohistological analysis, whilst the remaining samples were used for the isolation of urothelial cells. Once the urothelial cells were isolated they were cultured and maintained in a serum-free medium, using an adapted protocol from our well-established procedures for the culture of normal human urothelial cells. We have successfully established cultures of urothelial cells from two patients with IC (conforming with the NIDDK criteria) and three control patients using a modified culture protocol. We have demonstrated that the cells grow well in culture and can be passaged and maintained for comparable periods of time to cultures from normal human urothelium. Interestingly, we have found that in culture a proportion of cells isolated from IC urothelium seem to be enlarged and are multinuclear, which could be indicative of failed division. Using established in-vitro model systems, we are investigating induction of differentiation in the urothelial cells and their capacity to form a functional barrier.

Dual Innervation by Primary Afferents of Pelvic Visceral Organs

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POSTER 5

Irritable bowel syndrome (IBS) and interstitial cystitis (IC) are analogous disorders of pelvic visceral pain (PVP). The frequent overlap of IBS, IC, and other PVP syndromes suggests a common underlying mechanism. We have shown that acute pelvic organ irritation in rats leads to cross-organ sensitization via neuronal cross talk. Others have demonstrated the importance of visceral afferent anatomic distribution for response to peripheral stimuli. Thoracolumbar (TL) afferents become sensitized following inflammation, whereas lumbosacral (LS) afferents respond to acute mechanical stimuli. In this study, we performed concomitant retrograde labeling of urinary bladder (UB) and distal colonic (DC) afferents. Anesthetized SD rats underwent midline laparotomy, and 10 ul of Alexa Fluor 647-conjugated cholera toxin subunit B (CTB) was injected into the distal colon (2-4 cm from the anus) in 2-3 ul increments. Likewise, 10 ul of CTB conjugated to Alexa Fluor 488 was injected into the urinary bladder. Five days later the T10-S2 dorsal root ganglia (DRG) were removed and sectioned. Labeling was analyzed by confocal microscopy. Our analysis revealed that rat DC and UB afferents project to TL and LS spinal regions, specifically L1-L2 and L6-S1 DRG. Approximately 31% of DC and 36% of UB afferents projected to L1-L2 DRG. Significantly more labeling was observed in L6-S1 DRGs, comprising 53% of DC and 56% of UB afferents. Double-labeling was observed in 15% of all labeled DRG neurons. In contrast to afferents innervating a single organ, significantly more of these double-labeled afferents projected to L1-L2 (56%) than L6-S1 (26%). These findings suggest that the rat DC and UB have limited dual-innervation that projects to L1-L2 DRGs. Activation of these dual-target afferents may underlie the cross-organ sensitization observed in our previous studies of pelvic irritation.

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The Role of the NF-κB Signaling Pathway in the Pathogenesis of Interstitial Cystitis

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POSTER 6

Please see "Speaker Abstracts" section.

A Model for Characterization and Reepithelialization of Bladder Urothelium

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POSTER 7

Bladder urothelium serves as a barrier function between the urine and underlying mucosa. A feature of interstitial cystitis (IC) is the presence of epithelial defects which suggest an abnormal or compromised epithelial barrier. Under normal conditions, epithelial cells in vivo regenerate in response to injury. Part of this response involves reepithelialization in which cells from the edges of a wound migrate and grow inward as well as proliferate. We are currently in the process of developing a cell culture model to characterize cell junction proteins involved in the barrier function and the bladder epithelial response to injury under normal and IC conditions.

Human bladder epithelial cells are isolated from surgical samples, grown on glass slides, and characterized for cell junction proteins. As previously reported by Southgate et al *(Lab Invest 71:583, 1994)*, human urothelial cells grown in the presence of calcium stain for the adherens junction constituent, E-cadherin. Positive staining by fluorescent antibodies demonstrates the presence of tight junction proteins, ZO-1 and occludin in our system. Claudins represent a family of integral membrane proteins which comprise tight junctions. Immunohistochemical staining of cell cultures showed claudin 1 and 4 to be positive on more mature cells while no staining was evident with claudins 3 and 5.

As a model to examine repair of epithelial defects seen in IC, human bladder epithelial cells are grown to confluence on glass slides, wounded with a comb, and incubated for increasing time periods. Fixing and staining cells with H and E shows repair of the wounds to occur by both en masse migration as well as by individual cells moving into the wound. Further, cells on or near the edge of the wound appear to increase in area. With the use of microscopy and computer analysis (Image Pro Plus, MediaCybernetics, Silver Spring, MD), we have determined that cells decrease wound width at a rate of 17 ± 3 (SE) microns/hour.

Two prevalent etiologic theories of IC are that of a defect in the bladder epithelium and a pathophysiologic role for the mast cell. Mast cells release tryptase, which is elevated in the urine of IC patients. Future studies will include using our cell model system to look at cell morphology and rate of wound healing in the absence and presence of tryptase. Taken together, these results should increase our understanding to the response of bladder epithelial cells to injury in both the normal and IC bladder.

Time-Course of Mouse Urinary Cytokine Profile in Response to Intravesical Instillation With BCG, TNF-Alpha and LPS

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POSTER 8

Introduction:
Bacillus Calmette-Guerin (BCG) has been successfully applied to the treatment of superficial bladder cancer for over two decades and has been presented as a promising option for treatment of interstitial cystitis. However, the mechanisms underlying BCG therapy are not completely understood. In addition, intravesical BCG therapy induces bladder inflammation.

Objectives:
To determined the profile of cytokines in the mouse urine secondary to BCG treatment. In addition, the results obtained with BCG were compared with intravesical instillation of LPS and TNF-alpha.

Methods:
Ten to twelve weeks old C57BL/6 female mice were anesthetized, transurethrally catheterized, and instilled with 200 μl of one of the following substances: BCG (TheraCys®-Aventis-Pasteur; total dose of 1.35 mg), *E. coli* LPS (strain 055:B5; 100 μg/ml), or TNF-alpha (1 μg/ml) on days 1, 7, 14, and 21. Urine was collected at time 0 (previous to first instillation) and 7 days after each intravesical instillation. Urine for cytokine analysis was stabilized before freezing by the addition of a 10-fold concentrated buffer containing 2 M Tris-HCl (pH 7.6), 5% BSA, 0.1% sodium azide, and protease inhibitors. Cytokines were assayed in duplicates using Bio-Plex Mouse Cytokine 18-Plex (catalog #171-F11181, BioRad).

Results:
The primary chemokine released by BCG was KC which reached 47,000% above control levels. In addition to KC, BCG induced the release of the following cytokines: MIP-1α, IL-1α, RANTES, IL-6, G-CSF, IL-1β, IL-17, IL-4, IL-12(p70), and GM-CSF. In contrast, LPS induced the release of the following cytokines in decreased order: MIP-1α, IL-1α, G-CSF, KC, IL-1β, IFN-γ, and RANTES. TNF induced the following cytokines in decreased order: G-CSF, KC, IL-1α, IL-6, IL-2, IL-17, MIP-1α, IL-5, RANTES, IL-4, IFN-γ, and IL-1β. In terms of time, acute (24 hours) BCG induced IL-12(p70), IL4, and RANTES; IFN and TNF appeared at 7 days after a single instillation; IL5, IL-10, IL-3, IL6, IL12p40, G-CSF, KC. IL-5, and IL-10 after 14 days; and the remaining cytokines peaked at day 28. In contrast, LPS acutely (24 hrs) released KC and RANTES. TNF induced a peak release of GM-CSG at 24 hours; IL-5 at day 14; and the other cytokines peaked on day 28.

Conclusion:
The present experiment lays the groundwork for urinary cytokine profiling of the mouse and indicates a potent and differential effect of BCG when compared with LPS and TNF-alpha.

Visualization of Lymphatic Vessels Through Nf-κB Expression.

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POSTER 9

Objectives:
The characteristics of blood vascular versus lymphatic endothelium have remained poorly defined. Moreover, the specific pro- and anti-lymphangiogenesis factors still remain to be determined. The purpose of this work was to determine whether the urinary bladder blood and lymphatic vessels exhibit a basal NF-κB activity.

Methods:
kB-lacZ mice and Tie2-*lacZ* mice were used in these experiments. **κB-*lacZ* mice** harbor a fragment derived from the p105 promoter, which includes three functional kB sites and is inserted upstream of the gene encoding the *E. coli* β-galactosidase with a nuclear localization sequence, and act as a reporter for NF-κB activity (Development 1996, 122:2117-2128). **Tie2-*lacZ* mice** present *lacZ* expression driven by an endothelial-specific promoter/enhancer (Jackson Laboratories). Tissues were removed from naïve mice and analyzed as whole-mount and cross sections by X-gal staining alone or in combination with LYVE-1 antibody labeling (Dev Cell 2002, 3:411-423).

Results:
The urinary bladder, stomach, heart, colon, and uterus of κB-*lacZ* mice presented a clear pattern of X-gal and LYVE-1 labeling in the adventitia layer. This label was interrupted at intervals by constrictions, which gave them a knotted or beaded appearance, thus indicating a basal level of NF-κB activity in lymphatic vessels. Results obtained with Tie2-*lacZ* mice clearly revealed that the structures observed in κB-*lacZ* mice were not blood vessels. LYVE-1 immunohistochemistry of both whole-mounts and cross-sections confirmed that all lymphatic vessel endothelial cells displayed constitutive NF-κB activity.

Conclusions:
Our results indicated a basal NF-κB activity specifically in lymphatic vessels. In addition to the urinary bladder, NF-κB activity in lymphatics was observed in the heart, stomach, colon, and uterus. It remains to be determined how alterations in NF-κB pathway will alter lymphatic development in health and disease. In this context, the use of LYVE-1 antibody will permit the study of the lymphatic cell lineage during development and provide a tool for the study of pro- and anti-lymphangiogenesis factors.

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Prostaglandin Dehydrogenase (PGDH), a Novel Urothelium Differentiation Factor

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POSTER 10

Urinary bladder is lined with urothelial cells which are modestly stratified, non-keratinizing transitional epithelium, average 3-6 cells in depth. Division occurs most frequently in the basal layer. As urothelial cells differentiate, they migrate towards the luminal surface, resulting in the fact that the most superficial cells are most differentiated. Urothelial cells serves as a barrier between urine and the rest of the body. Urine is usually hyper-osmotic so could damage the cells of the bladder if allowed unlimited access. Furthermore, toxic materials and bacteria could also be present in urine. Failure to prevent them from entering into the body would have dire effects on the bladder and the rest of the body. The surface of the bladder is lined with urothelial cells which possess the capability to prohibit the access of these materials thus avoid such tragedies from happening.

Interstitial cystitis (IC) is a poorly understood syndrome that includes pain, frequent urination, and urinary urgency. There is increasing amount of evidence that differentiated urothelial cells are lost, along with the mature differentiated urothelial barrier function, in IC patients and spontaneous cat models of IC. Therefore, better understanding of the mechanism of urothelium differentiation could lead to better understanding of IC and potential treatment for it.

We have identified a panel of biomarkers associated with urothelial differentiation using differential display RT-PCR. Prostaglandin dehydrogenase (PGDH), a major enzyme responsible for degradation of bioactive prostaglandins, is further investigated in this report. Northern analysis confirmed the up-regulation of *PGDH* gene in differentiated urothelial cells and the anti-PGDH immunostaining also demonstrated increasing amount of PGDH expression in luminal cells (more differentiated) with lack of staining in undifferentiated basal cells. To examine the role of PGDH in urothelial cells and urothelial differentiation, siRNA assay was adopted to address the issue. We found that upon down-regulation of PGDH expression in RT4 cells (with only 10% PGDH remained), RT4 cells seemed to grow much slower than their control counterparts, which suggests that PGDH plays a role in the normal growth of urothelial cells. In addition, E-cadherin was found to localize predominately in the cytoplasm rather than on cell membrane, a sign of de-differentiation, in RT4 cells with significantly less amount of PGDH. These results could be suggesting a scenario where there is a dosage-dependent effect of PGDH in urothelial cells. These data suggest that PGDH is not only a promising biomarker for urothelial differentiation, but plays a functional role as well. Research is currently ongoing to test and further expand our working hypothesis of the role(s) of PGDH in urothelial cells.

Upregulation of Protease Activated-Receptors (PARs) in Urinary Bladder after Cyclophosphamide (CYP)-Induced Cystitis and Colocalization with Capsaicin Receptor (VR-1) in Bladder Nerve Fibers

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POSTER 11

Purpose:
Studies suggest that PARs are mediators of inflammation and repair. Recent studies suggest a neurogenic mechanism for PAR2 in inflammation and suggest an interaction between PAR2 and transient receptor vanilloid receptor (TRPV1 or VR1) being important for induction and maintenance of inflammatory pain.

Materials and Methods:
We examined expression of PARs (PAR2, PAR3, PAR4) in urinary bladder (urothelium and detrusor smooth muscle whole-mounts) of control, female rats and those treated with CYP acutely (4, 48 hr) or chronically (every 3rd day for 10 days) using Western blot and immunohistochemical techniques. Colocalization of PARs in nerve fibers and VR1-IR nerve fibers was determined by double-labeling techniques for protein gene product (PGP9.5) and capsaicin receptor (VR1). Bladder afferent cells in the dorsal root ganglia (DRG) were labeled by Fastblue injection into the urinary bladder smooth muscle 5-7 days prior to euthanasia. Complementary DNA was reversed transcribed from lumbosacral DRG total RNA and amplified using primers for PAR2-4.

Results:
Western blot revealed an upregulation (1.5-9.4-fold) of PAR2, PAR3 and PAR4 in the urinary bladder after cystitis. Immunohistochemistry revealed PAR2, PAR3 and PAR4 expression in the urothelium, detrusor muscle and nerve fibers. Confocal microscopy revealed colocalization of PAR2-4 with PGP9.5 and VR-1 suggesting that PARs are distributed in C-fiber bladder nerves. Bladder afferent cells expressed PAR2-4 immunoreactivity and lumbosacral DRG expressed PAR2-4 transcripts.

Conclusions:
These studies demonstrate that: (1) CYP-induced cystitis upregulates PAR2-4 expression in urinary bladder; (2) PAR2-4 is expressed in urothelium, detrusor muscle and bladder nerve fibers in control and CYP-treated rats; (3) urinary bladder C-fibers and bladder afferent cells in the DRG express PAR2-4. These results suggest an involvement of PARs in bladder inflammation that may contribute to altered sensory processing and reflex function with bladder inflammation.

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Simultaneous Evaluation of Urothelial Permeability, Sensitivity, and Voiding Function in the Mouse

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POSTER 12

Interstitial cystitis is characterized by reduced void volume and enhanced frequency; enhanced urothelial permeability has been reported.[1,2] Intravesical potassium chloride can produce intense discomfort in the clinic, but it is not clear whether this results from enhanced permeability or sensitivity of urothelial nerves, or both. It is also not known whether KCl-induced discomfort is attributable either to the ionic or osmolarity challenge, or some interaction.

Methods are now available for characterizing voiding function in normal and genetically altered strains of mice. Mice are placed in individual metabolism cages, each suspended over a wide pan resting on an electronic balance.[3] Currently, two squads of twelve animals can be studied simultaneously.[4] Urine and feces fall directly on this pan, and the balance reports its mass estimate 10 times per second. A retroreflective photocell detector reports beam breaks as the animal walks around the cage, yielding frequency and the frequency of prolonged inactivity ("pausing"). Netcams have been placed under some animals to record events as separate movies.[4] Implanted transponders report body temperature and identity and facilitate automated body weight recording. Thus, we routinely measure: void volume, frequency, urine production rate, void duration[4], peak and sustained uroflow rate,[4] hematuria, body temperature and weight, rate of beam interruption, frequency of pauses >60 or >300 sec, and proportion of session spent pausing >60 or >300sec.

It is now possible to assess urothelial permeability and sensitivity simultaneously in this setting. We documented that cyclophosphamide- or protamine-induced urothelial injuries enhanced fluorescein plasma levels in mice after intravesical administrations.[5] We developed methods for chronic intravesical cannulation of mice;[6] the mouse is awake and unrestrained because of a catheter tether, swivel and counterbalanced arm assembly. We have used animals so prepared to deliver sodium fluorescein in pH7.4 buffer directly to the bladder. A microanalytical method was developed to measure plasma concentration in 20ul samples. Saphenous vein blood samples are collected before and after each ~225 min experimental session without adverse effect. An increasing series of infusate concentrations (0.1, 0.3, 1.0 mg/ml) was delivered Monday, Wednesday, and Friday, followed by a decreasing series the following week. Plasma levels increased with increasing infusate concentrations. Mice differed in achieved plasma level, and there was an interaction between concentration and the sequence of treatment. After 1mg/ml, plasma levels are measurable for up to 3 days.

Simultaneous Evaluation of Urothelial Permeability, Sensitivity, and Voiding Function in the Mouse

Continued from previous page.

POSTER 12

Sensitivity to potassium chloride is assessed simultaneously with two pumps with ten syringes each. All syringes on both pumps are filled with 1mg/ml sodium fluorescein in saline; the syringes on the second pump also contain 500mM KCl. The pumps are programmed to deliver a constant total flow of 2ml/hour, with the output mixing before delivery to the animal. By varying the ratio of flow rates, we change KCl concentration in successive 75 minute periods, while maintaining a constant sodium fluorescein concentration.

Void volume decreases with increasing KCl concentration, thus measuring a shift in the concentration-effect function across time is possible. Regression permits estimation of the KCl concentration associated with a particular void volume, and its association (or lack thereof) with plasma fluorescein, an index of urothelial permeability. This strategy enables the assessment of the sensitivity of urothelial sensory function independently of the status of the urothelial permeability barrier in individual mice.

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