INFECTION AND IMMUNITY, Feb. 2011, p. 708–715 Vol. 79, No. 2
0019-9567/11/$12.00 doi:10.1128/IAI.01102-10

Autocrine Effects of Interleukin-6 Mediate Acute-Phase Proinflammatory and Tissue-Reparative Transcriptional Responses of Canine Bladder Mucosa[▽]

Michael W. Wood, Edward B. Breitschwerdt, and Jody L. Gookin*

Center for Comparative Medicine and Translational Research and Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, North Carolina 27606

Received 15 October 2010/Accepted 17 November 2010

During early urinary tract infection (UTI) the interplay between invading bacteria and the urothelium elicits a mucosal response aimed at clearing infection. Unfortunately, the resultant inflammation and associated local tissue injury are responsible for patient symptoms. Interleukin-6 (IL-6), a cytokine released during acute UTI, has both pro- and anti-inflammatory effects on other body systems. Within the urothelium, the IL-6 native-tissue origin, the target cell type(s), and ultimate effect of the cytokine on target cells are largely unknown. In the present study we modeled the UTI IL-6 response *ex vivo* using canine bladder mucosa mounted in Ussing chambers to determine the inflammatory and reparative role of IL-6. We demonstrated that uropathogenic *Escherichia coli* infection stimulates the synthesis of IL-6 by all urothelial cell layers, with the urothelial cells alone representing the only site of unequivocal IL-6 receptor expression. Autocrine effects of IL-6 were supported by the activation of urothelial STAT3 signaling and SOCS3 expression. Using exogenous IL-6, a microarray approach, and quantitative reverse transcriptase PCR (q-RT-PCR), 5 target genes (tumor necrosis factor alpha, interleukin-1β, matrix metallopeptidase 2, heparan sulfate D-glucosaminyl 3-*O*-sulfotransferase 3A1, and hyaluronan synthase 2) that have direct or indirect roles in promoting a proinflammatory state were identified. Two of these genes, heparan sulfate D-glucosaminyl 3-*O*-sulfotransferase 3A1 and hyaluronan synthase 2, are also potentially important mediators of wound repair via the production of glycosaminoglycan components. These findings suggest that IL-6 secretion during acute UTI may serve a dual biological role by initiating the inflammatory response while also repairing urothelial defenses.

Urinary tract infections (UTIs) are the second most common infection of the human body. Nearly 11% of women 18 years of age or older experience at least one UTI per year, with combined annual medical costs exceeding 1 billion dollars (6). Even patients with uncomplicated UTI experience on average 6.1 days with symptoms and 2.4 days of restricted activity; therefore, each episode can be debilitating (7). Given the prevalence, costs, and morbidity associated with these infections, there has been ongoing emphasis placed on an understanding of the mucosal response to UTI and how the response can be modified to improve clinical outcomes.

Paramount to UTI prevention is the maintenance of the complex multicellular urothelium that lines the bladder lumen. There are numerous urothelial elements that contribute to the prevention of the bacterial colonization of the bladder. Three of the most integral components include uroplakin plaques within the umbrella cell apical membranes (21, 30), tight junctions adjoining umbrella cells (18, 27), and a hydrophilic glycosaminoglycan (GAG)-rich mucus layer covering the luminal surface of the umbrella cells (26, 36). Collectively, these three components restrict the translocation of ions, solutes, and bacteria into the bladder interstitium and protect the urothelial microenvironment from injury. During urinary tract infections these defenses are compromised, and the urothelium initiates a local response that includes the release of cytokines, growth factors, and other inflammatory mediators (12, 41). This inflammatory response is both an asset and a detriment. The initial innate response is essential for the recruitment of phagocytic cells and the clearance of infection; however, inflammation is also associated with tissue injury resulting in clinical signs such as painful, frequent, and difficult urination (41). The urothelial balance of pro- and anti-inflammatory responses is therefore essential to bacterial eradication while minimizing tissue pathology.

The synthesis of interleukin-6 (IL-6) is an important component of the urinary bladder response to bacterial infection (2, 8, 14, 15); however, the target cell type(s) and functional effects of IL-6 within the urinary bladder are incompletely understood. Throughout the body, IL-6 is an important pleiotropic cytokine that promotes tissue homeostasis after acute injury and infection (9). Three well-described roles of IL-6 include cytoprotection, maintenance of the cellular microenvironment, and modulation of inflammation (1, 43, 45). All three of these actions could prove important to the maintenance and repair of damaged urothelial defenses during the acute phase of UTI. An enhanced understanding of the role of IL-6 during UTI may be vital to defining the role of therapeutic agents that enhance urothelial repair.

In the present study, we first characterized the IL-6 secretory response of canine bladder mucosa to uropathogenic *E. coli* infection *ex vivo* in Ussing chambers. We then recapitulated the concentration and location of IL-6 secretion in the absence

* Corresponding author. Mailing address: Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606. Phone: (919) 513-6295. Fax: (919) 513-6336. E-mail Jody_Gookin@ncsu.edu.

▽ Published ahead of print on 29 November 2010.

of *Escherichia coli* using exogenous canine IL-6. This approach facilitated sustained viability, the physical separation of suburothelial and lumen influences, and the isolation of the IL-6 response. As this model maintains viable intact tissue in the absence of a blood supply, the system is able to specifically isolate the action of IL-6 on the urothelium without the confounding effects of recruited inflammatory cells. Given these unique features, this approach allowed us to isolate changes in gene expression associated with IL-6 induction and tissue remodeling.

The central hypothesis of the described work contends that locally secreted IL-6 binds to urothelial membrane-associated IL-6 receptors and alters gene expression within the mucosa. Upregulated genes encode proteins that modify the inflammatory response, thereby enhancing the repair of the damaged urothelial microenvironment.

MATERIALS AND METHODS

Animals. Urinary tract infections in both dogs and humans mirror each other, with similar clinical signs caused primarily by uropathogenic strains of *E. coli* (3, 20, 40); hence, canine urinary bladders were chosen as the experimental tissue in our *ex vivo* model. Intact urinary bladders were obtained from beagle dogs (aged 6 months to 1 year; Covance Laboratories) immediately after intravenous sodium pentobarbital euthanasia. The urine sterility of each animal was confirmed by aerobic culture (10% blood agar for 14 days at 37°C) of urine samples aspirated from the bladder. All studies were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Ussing chamber parameters. Urinary bladders were bathed in an oxygenated Ringer's solution (154.1 mM Na^+, 6.3 mM K^+, 1.2 mM Ca^{2+}, 0.7 mM Mg^{2+}, 137.3 mM Cl^-, 24 mM HCO_3^-, 1.65 mM HPO_4^{2-}). Sterile Ringer's solutions were filtered (0.22 μm) and treated with antibiotics (streptomycin, 50 μg/ml; penicillin, 50 IU/ml). *E. coli*-infected Ringer's solutions were prepared similarly; however, antibiotics were omitted. The urinary bladder was bisected longitudinally, and the seromuscular and submucosal layers were removed by sharp dissection. The resulting mucosal sheets (urothelium and lamina propria) were mounted in 3.14-cm^2-aperture Ussing chambers, and both surfaces were bathed with Ringer's solution containing glucose (10 mM submucosal) and mannitol (10 mM lumen). Solutions were oxygenated (95% O_2, 5% CO_2), circulated by gas lift, and maintained at 37°C by water-jacketed reservoirs.

Bacteria. Uropathogenic *E. coli* J96, originally isolated from a human patient with pyelonephritis (22) (kindly supplied by Paul Orndorff, North Carolina State University), was grown to log phase at 37°C in Luria-Bertani broth and washed three times in Ringer's solution prior to addition to the lumen reservoir of Ussing-chambered bladder mucosae to achieve a final concentration of 1×10^8 CFU/ml. This concentration of *E. coli* was chosen based on previously reported work demonstrating that *E. coli* concentrations of 1×10^8 CFU/ml and 1×10^9 CFU/ml were able to stimulate IL-6 secretion *in vivo* and *in vitro*, respectively (15, 17).

Exogenous interleukin-6. Recombinant canine IL-6 (R&D Systems, Minneapolis, MN) was added to the submucosal reservoir of Ussing-chambered bladder mucosae at a concentration of 20 ng/ml. This recapitulated the 5-h submucosal reservoir concentration of IL-6 induced by the *ex vivo* infection of the bladder mucosa with uropathogenic *E. coli* and falls within the range of IL-6 levels measured in humans with naturally occurring UTI (23).

Immunofluorescence microscopy. After removal from the Ussing chamber, mucosae were embedded in optimal-temperature cutting medium and frozen-sectioned at a 4-μm thickness. Sections were fixed in 100% ethanol and blocked with 1% (vol/wt) bovine serum albumin (BSA) and 2% (vol/vol) goat serum in PBS^+ (1× phosphate-buffered saline [PBS], 0.12% 1 M $CaCl_2$ [pH 7.4]) for 1 h at 4°C prior to incubation with primary antibodies. Primary antibodies (1:50 in blocking buffer) were applied for 1 h at room temperature and included biotinylated polyclonal goat anti-canine IL-6 and biotinylated polyclonal goat anti-human soluble IL-6 receptor (R&D Systems, Minneapolis, MN). Fluorescence labeling was performed by using streptavidin-conjugated Alexa Fluor 488 (1:100; Invitrogen, Eugene, OR) and FITC-labeled control mouse IgG1 (1:500; BD Pharmingen, Franklin Lakes, NJ) for 30 min at room temperature. Sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and visualized by using an epifluorescence microscope. For the demonstration of cytokine synthesis, bladder mucosae were treated with the Golgi protein transport inhibitor monensin (1 μl/ml of 1.5× solution; BioLegend, San Diego, CA) applied to both the lumen and submucosal reservoir of the Ussing chamber.

IL-6 ELISA. A canine-specific IL-6 chemiluminescence enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) was used to assay IL-6 in duplicate samples obtained from the lumen and submucosal reservoirs bathing the bladder mucosa. Components of the ELISA included capture (800 ng/ml) and detection (25 ng/ml) goat anti-canine polyclonal IL-6 antibodies, streptavidin-horseradish peroxidase (HRP) (1:200 in Femto Luminol/Enhancer), and Femto stable peroxide substrate (Pierce, Woburn, MA). Recombinant canine IL-6 (R&D Systems, Minneapolis, MN) was used to create the standard curve, and results were reported as pg of IL-6 per ml Ringer's solution.

Western blot analysis. Soluble protein was extracted from liquid nitrogen-frozen samples of bladder mucosa by homogenization (Mini-Beadbeater; BioSpec Products, Bartlesville, OK) with stainless steel beads (3.2 mm) in radioimmunoprecipitation assay (RIPA) buffer (25 mM Tris-HCl, 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, 1% EDTA [pH 7.6]) containing antiproteases (1% each of Halt antiprotease cocktail [Pierce, Woburn, MA] and anti-phosphatase inhibitor cocktails 1 and 2 [Sigma-Aldrich, St. Louis, MO]). Equal protein concentrations, measured by a BCA assay, were electrophoretically separated in 4 to 12% Bis-Tris gradient gels (Invitrogen, Carlsbad, CA), transferred onto nitrocellulose, and sequentially immunoblotted using anti-phosphorylated STAT3 (pSTAT3) (Ser727) (0.5 μg/ml; Assay Designs, Ann Arbor, MI) and anti-total STAT3 (1:2,500; BD Biosciences, San Jose, CA) monoclonal antibodies or goat anti-human polyclonal anti-SOCS3 (1 μg/ml; Life Span Biosciences, Seattle, WA). Secondary reagents included anti-mouse IgG-HRP (1:10,000; Santa Cruz, Santa Cruz, CA) or donkey anti-goat IgG-HRP (1:10,000; Santa Cruz, Santa Cruz, CA), respectively, and a chemiluminescence substrate (Supersignal West Pico; Pierce, Woburn, MA). A pervanadate-treated HepG2 cell lysate (Axxora, San Diego, CA) was included as a positive control for pSTAT3, and a MOLT-4 cell lysate (Santa Cruz, Santa Cruz, CA) was used as a positive control for SOCS3. For the STAT blots, protein loading was normalized by calculating the pSTAT3-to-STAT3 ratio. For the SOCS3 blots, equal protein loading was confirmed by the immunoblotting of cell lysates with monoclonal anti-mouse actin (1:2,000; Chevicon, Billerica, MA), followed by secondary anti-mouse IgG-HRP antibodies (1:5,000; Santa Cruz, Santa Cruz, CA). Densitometry was performed by using SigmaScan software (Systat, San Jose, CA) and is reported as pSTAT3 normalized to total STAT3 or simply SOCS3.

RNA extraction. Approximately 100 mg of chambered bladder mucosa was placed into 600-μl aliquots of RLT lysis buffer (Qiagen, Valencia, CA) and flash-frozen in liquid nitrogen for storage at −20°C prior to extraction. Tissues were homogenized using two 3.2-mm stainless steel beads (BioSpec Products, Bartlesville, OK) agitated with a Mini-Beadbeater (BioSpec Products, Bartlesville, OK). RNA was extracted from the resultant suspension by using the RNeasy Plus minikit (Qiagen, Valencia, CA) according to the manufacturer's recommendations and stored at −80°C. RNA purity and quality were assessed by using an Agilent Bioanalyzer 2100 (Hewlett Packard, Corvallis, OR). RNA integrity numbers (RIN) for all samples met or exceeded 8.80.

Microarray. The RNA samples were amplified by using an Affymetrix GeneChip one-cycle cDNA synthesis kit and labeled by using an Affymetrix GeneChip IVT labeling kit (Affymetrix, Santa Clara, CA). The cRNA was hybridized to GeneChip Canine Genome 2.0 arrays (Affymetrix, Santa Clara, CA) containing probes for >20,000 genes. The images were captured by using an Affymetrix GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, CA).

The data were analyzed via principal-components analysis (PCA) to first identify outlier samples. A repeated-measures permutation analysis of differential expression (RM-PADE) was then utilized in combination with an overlap analysis to identify target genes. Genes were required to have a minimum of a 2-fold increase in levels of transcripts over control RNA, a P value of less than 0.05, and concordance in gene expression for all three dogs to be considered for further analysis.

q-RT-PCR. Seven target genes were validated via quantitative reverse transcriptase PCR (q-RT-PCR) using the above-mentioned extracted RNA. Total RNA was reverse transcribed with random hexamers (Applied Biosystems, Carlsbad, CA) using the Superscript II RT enzyme kit (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. Using a Bio-Rad ICycler, 100 ng of converted cDNA was amplified by using 100 nM both forward and reverse primers and SYBR green PCR master mix (Applied Biosciences, Framingham, MA). Primer pairs (Table 1) were designed by using the canine predicted base sequences found in the NCBI database. To ensure that cDNA and not genomic DNA was amplified, all primers were amplified across at least one intron-exon junction. Products of the q-RT-PCR were validated by gel electrophoresis to confirm product sizes. The product melting temperature and se-

TABLE 1. Primer sequences, product sizes, and melting temperatures used for q-RT-PCR of designated target genes

Gene[a]	GenBank accession no.	Primer sequence (5′→3′)	Product size (bp)	Product T_m[b]
HPRT	NM_001003357	CGC TGA GGA TTT GGA AAA AG AAT CCA GCA GGT CAG CAA AG	150	82.5
TNF-α	NM_001003244	TGC CTG CTG CAC TTT GG GCT ACT GGC TTG TCA CTT GG	125	84.0
MMP-1	XM_546546	CGC GTA AAT CCC TTC TAT CC CAT CCT GAC CCT GAA CAA CC	157	80.5
MMP-2	XM_535300	TGG AGC AAG AAC AAG AAG ACC CCC TTG AAG AAG TAG CTA TGA CC	182	82.0 + 85.5
MMP-3	NM_001002967	ACA CCA GCT GCA TGT GAC C GAA CCC AGG TTC AAG TGT CC	123	82.0
HS3ST3A1	XM_546631	TCA TCG GCG TGA AGA AGG GCG GGT GAC AAA GTA ACT GG	200	91.0
IL-1β	NM_001037971	CAG GAC ATA AGC CAC AAA TAC C CAA AGC TCA TGT GGA ACA CC	109	80.0
HS2	XM_539153.2	TTC AGA CAC CAT GCT TGA CC TCT CAC ACT GCT GAG GAA GG	142	59.5

[a] HPRT, hypoxanthine phophoribosyltransferase, TNF-α, tumor necrosis factor alpha; MMP-1, matrix metalloproteinase 1; MMP-2, matrix metallopeptidase 2; MMP-3, matrix metallopeptidase 3; HS3ST3A1, heparan sulfate D-glucosaminyl 3-*O*-sulfotransferase 3A1; IL-1β, interleukin-1β; HS2, hyaluronan synthase 2.
[b] T_m, melting temperature.

quence were also verified (Davis Sequencing, Davis, CA). The q-RT-PCR negative control was a q-RT-PCR that included no template. Relative fold changes in gene transcription ($2^{-\Delta\Delta CT}$) were determined by using the hypoxanthine phosphoribosyltransferase (HPRT) canine housekeeping gene and comparative threshold cycle (C_T) analysis as described by ABI Prism 7700 sequence detection system user bulletin 2.

Statistical analysis. Statistical analyses were performed by using commercially available software (SigmaStat software; Systat, San Jose, CA). Values are reported as means ± standard errors. Data were tested for normality and analyzed by using parametric or nonparametric tests where appropriate. Parametric data were analyzed by using the Student *t* test. Nonparametric data were analyzed by using a Mann-Whitney rank sum test. Data were paired when appropriate. In all cases, *n* equals the number of dogs.

RESULTS

Uropathogenic *E. coli* stimulates basolateral secretion of interleukin-6 by the urothelium. Urothelial cells in culture will secrete IL-6 in response to infection with *E. coli* (2, 8, 14), and bacterial urinary tract infection *in vivo* results in increases in urinary IL-6 concentrations (15). Using intact canine bladder mucosae, we sought to first establish if the *E. coli*-induced synthesis of IL-6 is confined to the urothelium and whether the primary secretion is selectively directed to either the lumen or lamina propria of the bladder. Five hours after the inoculation of *E. coli* J96 (1×10^8 CFU/ml) into the luminal reservoir of the Ussing chamber containing bladder mucosa, significant increases in concentrations of IL-6 were measured in the submucosal reservoir but not in the luminal reservoir (Fig. 1). The identification of IL-6-secreting cells by means of immunofluorescence demonstrated that IL-6 synthesis was restricted to the urothelial cells, with no apparent contribution by cells residing in the underlying lamina propria. Moreover, all layers of the urothelium were identified as participating in the synthesis of IL-6 (Fig. 2A).

The urothelium is an autocrine recipient of IL-6 signals in response to uropathogenic *E. coli* infection. While the synthesis of IL-6 is a salient response of the urinary bladder to bacterial infection, the target cell type(s) and functional effects of IL-6 remain unknown. To identify the cellular target(s) of IL-6, we located IL-6 receptor-expressing cells by means of immunofluorescence. In both uninfected and *E. coli*-infected bladder mucosa, the constitutive expression of IL-6 receptors was confined to the urothelial cell layer. There was little evidence to support IL-6 receptor expression by cells residing within the underlying lamina propria (Fig. 2B). A nonspecific binding of antibody was not observed for tissues treated with nonimmune mouse IgG1 (Fig. 2C). We next examined if IL-6-mediated signaling pathways were activated within the urothelium in response to *E. coli* exposure. Accordingly, uninfected and *E. coli*-infected urothelia were removed from the Ussing chamber after 5 h, and the cellular lysates were immunoblotted for total and phosphorylated STAT3. A significant phosphorylation of STAT3 was observed for urothelia infected with *E. coli* compared to uninfected urothelia, indicating that an IL-6-depen-

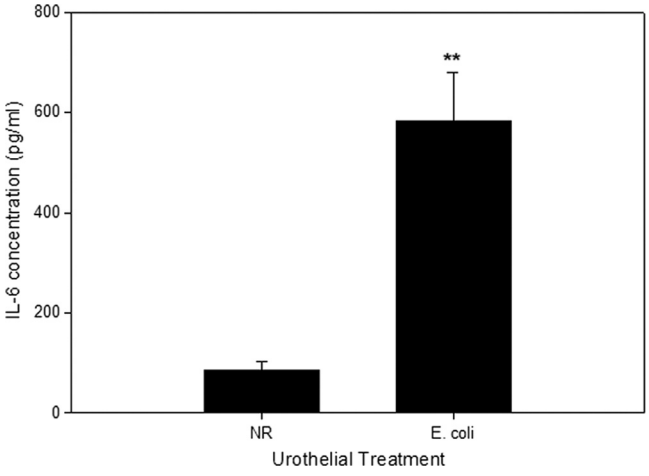

FIG. 1. Secretion of IL-6 by urothelium in response to normal Ringer's solution (NR) alone or luminal infection with uropathogenic *E. coli* J96 (1×10^8 CFU/ml). IL-6 concentrations were measured in the submucosal reservoir of the Ussing chamber after 5 h of infection. IL-6 was below the limit of detection in the lumen reservoir (data not shown). **, $P < 0.01$ ($n = 6$ dogs for each treatment condition).

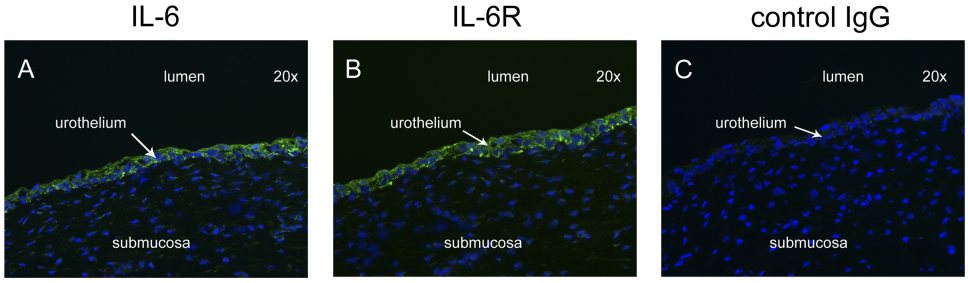

FIG. 2. Immunofluorescence localization of IL-6 and IL-6 receptors in *E. coli*-treated canine urothelium. Urothelia mounted in Ussing chambers were exposed to 1×10^8 CFU/ml of *E. coli* J96. The tissue was simultaneously exposed to monensin. (A and B) Both IL-6 (A) and IL-6 receptors (IL-6R) (B) were restricted in location to the urothelial cells. (C) Fluorescence was not observed in sections incubated with mouse IgG1 control antibodies.

dent signaling pathway was activated in the *E. coli*-treated tissues (Fig. 3).

Exogenous IL-6 is sufficient to activate urothelial gene transcription. As a prelude to examining the isolated effect of IL-6 on urothelial gene expression, we first examined whether exogenous IL-6 alone was sufficient to activate urothelial STAT3 signaling in a manner similar to that observed with *E. coli*. Accordingly, tissue was exposed to normal Ringer's solution (NR) or exogenous canine recombinant IL-6 at a polarity and a concentration that recapitulated *E. coli*-induced IL-6 secretion. After 5 h of treatment, protein lysates were prepared from the harvested urothelium and immunoblotted for total and phospho-STAT3. Unexpectedly, there was no difference in STAT3 phosphorylation between the IL-6- and NR-treated tissues ($P = 0.12$).

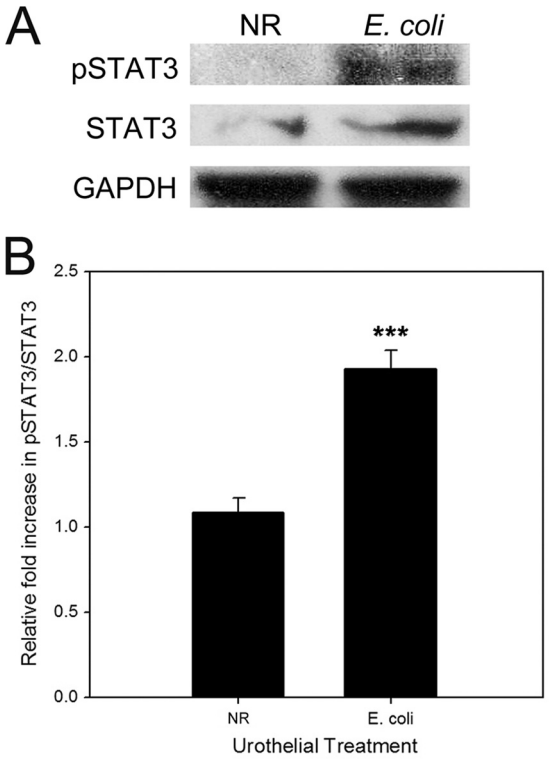

FIG. 3. Infection of canine urothelium with *E. coli* promotes phosphorylation of STAT3. After a 5-h treatment in Ussing chambers with normal Ringer's solution (NR) or uropathogenic *E. coli* J96 (1×10^8 CFU/ml), canine bladder mucosae were immunoblotted for phosphorylated STAT3 (pSTAT3), total STAT3, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (protein loading control). *E. coli* significantly increased the phosphorylation of STAT3 compared to uninfected tissue (normal Ringer's solution). Densitometric data represent relative fold increases in pSTAT3 compared to total STAT3. ***, $P < 0.001$ ($n = 4$ dogs each).

In contrast to *E. coli*, which must first stimulate IL-6 secretion before STAT signaling is activated, exogenous IL-6 activates STAT signaling immediately. Therefore, we were suspicious that negative regulatory factors such as SOCS3 may have reduced pSTAT3 concentrations in the urothelial cells after 5 h of direct exposure to IL-6. Since pSTAT3 induces the transcription of SOCS3, the demonstration of an increased expression of SOCS3 would support this observation as well as urothelial gene expression in response to the exogenously added IL-6.

Predictably, urothelia mounted within Ussing chambers and exposed to exogenous IL-6 had significantly more SOCS3 protein detected by immunoblotting than the NR control ($P < 0.05$) (Fig. 4). These results support the hypothesis that exogenously added IL-6 activates STAT3 signaling and SOCS3 expression and confirmed that exogenous IL-6 is sufficient to mediate urothelial cell transcription during the 5-h experimental timeframe.

Differential urothelial gene expression induced by IL-6. To determine the identity of urothelial genes whose transcription is induced by IL-6, sheets of canine urothelium were treated with exogenous canine recombinant IL-6 or the NR control for a period of 5 h, after which the tissue was harvested from the Ussing chambers and the RNA was extracted. Comparing gene lists generated from PCA, overlap, and random-measure statistical analyses, a total of 49 common genes had ≥2-fold increases (Table 2) or decreases (Table 3) in gene expression, with concordant results for all dogs, and a P value of <0.05. Seven target genes important for acute inflammation and tissue remodeling were selected for verification by q-RT-PCR, including tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), matrix metalloproteinase 1 (MMP-1), matrix metallopeptidase 2 (MMP-2), matrix metallopeptidase 3 (MMP-3), heparan sulfate D-glucosaminyl 3-*O*-sulfotransferase 3A1 (HS3ST3A1), and hyaluronan synthase 2 (HS2). Increases in levels of gene transcription (fold change ± SE) were confirmed by q-RT-PCR as statistically significant for TNF-α (3.98 ±

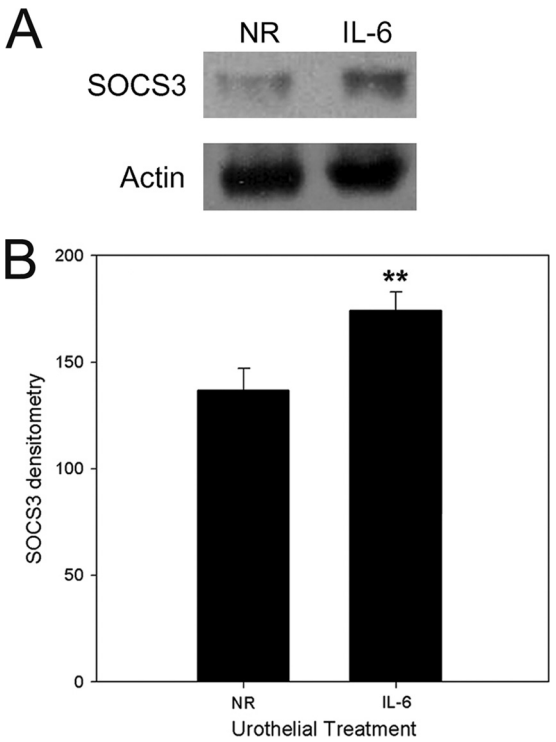

FIG. 4. Exogenous IL-6 activates urothelial cells and initiates transcription. After a 5-h treatment in Ussing chambers with normal Ringer's solution (NR), exogenous IL-6 canine bladder mucosae were immunoblotted for SOCS3 and actin (protein loading control). Exogenous IL-6 significantly increased SOCS3 production compared to untreated tissue (NR). **, $P < 0.05$ ($n = 4$ dogs each).

1.40), IL-1β (3.20 ± 0.85), MMP-2 (2.79 ± 0.41), HS3ST3A1 (4.40 ± 1.15), and hyaluronan synthase 2 (2.81 ± 0.66) compared to tissue treated with NR alone ($P < 0.05$).

DISCUSSION

A key biological response of the bladder to urinary tract infection is the secretion of IL-6 (15). While not normally present in the urine (16), IL-6 is commonly found in the urine of patients with cystitis or pyelonephritis (16, 32) and appears in human urine within 4 h of experimental infection with *E. coli* (15). Using cell culture models, previous studies have shown that urothelial cells secrete IL-6 in response to infection with *E. coli* (2, 8, 14). Whether IL-6 synthesis is confined to the urothelium, is secreted directly into the urine, or translocates into the urine secondary to an increase in bladder permeability is unknown. By clarifying the source and polarity of IL-6 secretion in the context of the entire bladder mucosa, the present studies have provided insights into the cellular targets and function of this cytokine.

Using an *ex vivo* preparation of canine bladder mucosa, these studies demonstrate that IL-6 is synthesized by all layers of the urothelium and is secreted exclusively into the underlying lamina propria but was not secreted into the luminal chamber after exposing the urothelium to *E. coli*. Whereas elevations in urine IL-6 concentrations were measured within hours of experimental urinary tract infection in humans (15), similar elevations were not detected during the same time frame in our *ex vivo* studies. A key difference between the two models is the absence of recruited inflammatory cells *ex vivo* compared to the immunologically intact host. These observations suggest that IL-6 may secondarily leak into the urine in association with the influx and transmigration of neutrophils (4). This may further explain why urine IL-6 concentrations appear to correlate with clinical disease severity, as the inflammatory process may be responsible for disrupting barrier function, resulting in the leakage of IL-6 from the urothelium (32).

While IL-6 is a predominant cytokine secreted by the urothelium in response to urinary tract infection, the cellular target and biological responses mediated by IL-6 in this microenvironment have remained poorly understood. In this Ussing chamber model, the urothelium was the only site of unequivocal IL-6 receptor expression within the bladder mucosa. As such, urothelial cells appear to be key autocrine recipients of IL-6 signals, an assertion which is supported by our demonstration of increased urothelial STAT3 signaling and SOCS3 expression. However, a direct effect of *E. coli* on STAT3 activation cannot be ruled out.

A local autocrine response of the urothelium to IL-6 is a potentially important observation. Despite being a frequent topic of research investigations, a consensus regarding the precise role of IL-6 in the various epithelia of different body systems remains ill defined. Generally, during acute injury, IL-6 has been associated with an attempt to return the tissue to a state of homeostasis. For both the epidermis and respiratory epithelium, IL-6 has been identified as being important for promoting tissue barrier repair after injury (44, 45). In models of endotoxic lung injury and endotoxemia, the presence of IL-6 is associated with decreased TNF-α concentrations and neutrophil infiltration (28, 46). Increased concentrations of IL-6 have also been found to promote the production of anti-TNF-α and anti-IL-1β molecules to modulate the inflammatory response (1, 39, 42). In contrast, IL-6 has been associated with proinflammatory actions such as increased neutrophil recruitment during *E. coli* pneumonia and when lung tissue is exposed to certain Gram-positive cell wall components such as peptidoglycan (PepG) (24, 25, 37). Given these seemingly opposing effects, the cellular role of IL-6 within different tissues should be investigated further. If applicable to other organ systems, our findings are important for the ongoing development and use of pharmaceuticals that alter IL-6 responses (31, 38).

Because patient morbidity during UTI is associated with the inflammatory response to bacteria (41), an understanding of the extent to which IL-6 augments or blunts this response is essential. By specifically targeting the intact urothelium using the Ussing chamber model, the role of IL-6 in gene transcription and protein expression can be studied. Using microarrays and q-RT-PCR, 5 genes that appear to play a role in IL-6-induced urothelial inflammation were identified.

In contrast to the effects observed for other tissues, IL-6 upregulated the transcription of genes in the urothelium that are responsible for both TNF-α and IL-1β synthesis. These two cytokines are known proinflammatory molecules with important functions during the acute-phase immune response, including promoting the migration of inflammatory cells and the production of cyclooxygenase-2, type 2 phospholipase A, and inducible nitric oxide synthase (5). These roles argue against the proposi-

TABLE 2. IL-6-dependent urothelial transcriptome upregulation

Functional category and Affymetrix ID	Fold change	Max *P* value	Gene description
Cytokine/chemokine response			
Cfa.54.1.S1_s_at	4.73	0.000060	Tumor necrosis factor
CfaAffx.11741.1.S1_s_at	2.92	0.000040	Interleukin 1 beta
Cfa.3554.1.S1_at	2.78	0.000040	Interleukin 1 beta
CfaAffx.21295.1.S1_at	2.84	0.000040	Chemokine (C-C motif) receptor-like 2
Cfa.14352.1.A1_at	2.68	0.000040	Chemokine (C-C motif) ligand 3
Cfa.15815.1.S1_s_at	2.05	0.000080	Chemokine (C-C motif) ligand 3
Cfa.16590.1.S1_s_at	2.36	0.000040	Chemokine (C-X-C motif) ligand 10
Cfa.16590.1.S2_at	2.22	0.000040	Chemokine (C-X-C motif) ligand 10
CfaAffx.1705.1.S1_at	2.20	0.000046	Gamma interferon-inducible protein 47
Cfa.3528.1.S1_s_at	2.14	0.000156	Interleukin-6
Matrix metallopeptidases			
Cfa.3447.1.S1_s_at	3.28	0.000040	Matrix metallopeptidase 3
CfaAffx.23166.1.S1_s_at	3.10	0.000040	Matrix metalloproteinase 1
CfaAffx.23166.1.S1_at	2.62	0.000040	Matrix metalloproteinase 1
Cfa.3597.1.S1_at	2.13	0.000260	Matrix metallopeptidase 2
CfaAffx.14851.1.S1_s_at	2.03	0.000046	Matrix metallopeptidase 2
Mediators of cell adhesion			
Cfa.3868.1.S1_at	3.20	0.000040	Selectin E
CfaAffx.23326.1.S1_s_at	2.51	0.000040	Selectin E
CfaAffx.23464.1.S1_s_at	3.00	0.001104	Dermatopontin precursor
Cfa.6133.1.S1_at	2.02	0.000040	Dermatopontin precursor
CfaAffx.13511.1.S1_at	2.93	0.000040	Immunoglobulin superfamily, member 10
Cfa.3983.1.A1_s_at	2.45	0.000260	Immunoglobulin superfamily, member 10
Glycosaminoglycan production			
CfaAffx.27384.1.S1_s_at	2.46	0.000054	Heparan sulfate D-glucosaminyl 3-*O*-sulfotransferase 3A1
CfaAffx.2351.1.S1_at	2.14	0.000334	Hyaluronan synthase 2
CfaAffx.24924.1.S1_at	2.38	0.000060	Colony-stimulating factor 3 isoform b precursor
CfaAffx.4373.1.S1_s_at	2.21	0.000040	Bone morphogenetic protein 5 precursor (BMP-5)
Cfa.9659.1.A1_at	2.16	0.000104	Vascular smooth muscle cell growth-promoting factor
Growth and differentiation factors			
Cfa.12671.1.A1_at	2.14	0.000546	Signaling lymphocyte activation molecule family member 7
CfaAffx.5498.1.S1_at	2.12	0.000040	CXCL7; leukocyte-derived growth factor; macrophage-derived growth factor
CfaAffx.18191.1.S1_s_at	2.11	0.002402	Myeloid cell nuclear differentiation antigen
CfaAffx.5548.1.S1_at	2.10	0.000546	Epiregulin
Cfa.3484.1.S1_at	2.05	0.002972	Progesterone receptor
Negative regulators			
CfaAffx.1046.1.S1_at	2.34	0.002972	Urokinase inhibitor
CfaAffx.2230.1.S1_s_at	2.31	0.000040	Thrombospondin 2 precursor
CfaAffx.1632.1.S1_s_at	2.26	0.000040	Radiation-inducible immediate-early gene IEX-1
CfaAffx.13597.1.S1_s_at	2.09	0.000614	Chondroitin sulfate proteoglycan 2
CfaAffx.25712.1.S1_at	2.02	0.000040	Jumonji domain-containing 3
Miscellaneous			
CfaAffx.13553.1.S1_at	2.83	0.001932	Succinate receptor 1
Cfa.13912.1.A1_at	2.35	0.000104	Ribonucleotide reductase small chain
Cfa.3973.1.A1_at	2.35	0.000984	Proteasome alpha 3 subunit isoform 2
Cfa.1370.1.A1_at	2.33	0.000040	Solute carrier family 2
Cfa.825.1.S2_at	2.10	0.000040	Solute carrier family 2
CfaAffx.13491.1.S1_at	2.22	0.000482	G-protein-coupled receptor 171
CfaAffx.4930.1.S1_s_at	2.21	0.000070	Alpha 1 type XII collagen short-isoform precursor
CfaAffx.1247.1.S1_s_at	2.18	0.000040	Vanin 1
Cfa.6170.1.A1_at	2.09	0.001384	GMP-PDE gamma[a]
CfaAffx.14532.1.S1_at	2.08	0.000040	Nuclear receptor subfamily 4, group A, member 2
Cfa.1416.1.A1_at	2.03	0.000876	Proenkephalin A precursor
Cfa.3589.1.S1_s_at	2.03	0.000070	Caspase 4, apoptosis-related cysteine peptidase

[a] PDE, phosphodiestrase.

tion that IL-6 is an anti-inflammatory cytokine within the urothelium. While it is generally accepted that these two early-response cytokines are responsible for inducing local IL-6 production (29), a direct effect of IL-6 on TNF-α and IL-1β expression would be a novel observation and suggests a urothelial positive-feedback circuit that promotes tissue inflammation.

TABLE 3. IL-6-dependent urothelial transcriptome downregulation

Functional category and Affymetrix ID	Fold change	Max *P* value	Gene description
Cytoskeleton			
CfaAffx.23771.1.S1_s_at	4.03	0.000040	Desmin
Cfa.45.1.S2_at	3.67	0.000614	Desmin
Cfa.12195.5.S1_at	3.15	0.000080	Actin
CfaAffx.11658.1.S1_s_at	2.97	0.002972	Myosin binding protein C
CfaAffx.835.1.S1_s_at	2.87	0.001236	Myosin regulatory light chain 2
Cfa.12198.1.A1_at	2.40	0.000080	Myosin regulatory light chain 2
Cfa.12841.1.S1_at	2.11	0.000334	Similar to alpha-1-syntrophin
Transport proteins			
CfaAffx.24259.1.S1_at	2.28	0.000692	Mitochondrial 2-oxoglutarate/malate carrier protein
Cfa.305.1.A1_at	2.04	0.000294	Heat shock 22-kDa protein 8
Cfa.305.1.A1_at	2.04	0.000294	Heat shock 22-kDa protein 8
Miscellaneous			
Cfa.9045.1.A1_s_at	2.72	0.000228	EF hand calcium binding domain 1
CfaAffx.24807.1.S1_at	2.54	0.000692	Glutathione peroxidase-related protein 2
Cfa.12202.1.A1_at	2.33	0.000040	Glutathione peroxidase-related protein 2
Cfa.17108.1.S1_at	2.41	0.000544	Mesothelin isoform 1 preproprotein

The upregulation of the MMP-2, HS3ST3A1, and HS2 genes provides additional evidence supporting positive-feedback effects of IL-6 within the urothelium. MMP-2 converts the IL-1β precursor to its active form (5, 10). Increasing concentrations of IL-1β would augment the inflammatory response within the epithelium. Heparan sulfate D-glucosaminyl 3-*O*-sulfotransferase 3A1 is an enzyme responsible for the synthesis of heparan sulfate. Heparan sulfate is a glycosaminoglycan carbohydrate that during inflammatory processes interacts with heparan binding proteins to play integral roles in leukocyte extravasation and chemotaxis as well as promote the release of the above-mentioned inflammatory cytokines TNF-α and IL-1 from macrophages (11). The HS2 gene also synthesizes a glycosaminoglycan carbohydrate, hyaluronan. As such, HS2 may play a role similar to that of HS3ST3A1, as hyaluronan has been associated with the increased macrophage production of IL-1 (19).

The importance of the IL-6-mediated upregulation of the HS3ST3A1 and HS2 genes is not limited to the production of proinflammatory cytokines. Both of these genes have potentially important effects on wound repair. As stated above, these enzymes are responsible for the production of glycosaminoglycan components. In the urinary bladder GAG serves a particularly important role in tissue defense as a mucus-rich GAG layer that lines the luminal surface of the bladder (35). This anionic layer is hydrophilic and traps water, creating a wall of water between the urothelial cells and the urine, which contains cationic solutes and potentially bacteria (33). Prior research demonstrated that a disruption of the GAGs will increase epithelial cell permeability (34). IL-6-induced heparan sulfate and hyaluronan production may represent a preemptive effort to repair a damaged GAG layer. If the increased production of these components can be linked to GAG layer repair, it would seem probable that they may also increase barrier function. Treatment with GAG replacers such as pentosan polysulfate sodium and chondroitin sulfate has shown promise in relieving pain and discomfort associated with interstitial cystitis as well as preventing bacterial adherence and barrier repair (13, 33). Therefore, a potential role for IL-6 in increasing GAG production during infection is a potentially important observation that may provide an alternate pathway for targeting therapy.

In conclusion, by isolating the urothelial microenvironment from recruited inflammatory cells, the present studies demonstrate that the inoculation of uropathogenic *E. coli* results in the synthesis of IL-6 by all urothelial layers, with selective secretion into the lamina propria. Furthermore, the urothelium was the only site of unequivocal IL-6 receptor expression identified within the resident bladder mucosa. In addition, autocrine effects of IL-6 were supported by the activation of urothelial STAT3 signaling and SOCS3 production. IL-6 also mediated the expression of genes responsible for the production of TNF-α and IL-β through both direct and indirect mechanisms. Besides promoting the synthesis of proinflammatory cytokines, the upregulation of the HS3ST3A1 and HS2 genes by IL-6 suggests a possible role for IL-6 in urothelial tissue repair and as a defense against invading bacteria. At this time, the latter evidence remains circumstantial.

Further application of the Ussing chamber model is likely to provide an important and relevant tool for identifying mechanisms of *E. coli* urothelial pathogenesis and for establishing the role of IL-6 in promoting or ameliorating the urothelial immune response. The model facilitates the isolation of the urothelial response to IL-6 following bacterial infection without the confounding effects of inflammatory cells. Future assessments of the bacterial/cytokine interplay and how manipulating the IL-6 response affects urothelial defense against bacterial colonization are crucial to our understanding of the mucosal response to UTI.

ACKNOWLEDGMENTS

Michael W. Wood was supported by the Ruth L. Kirschstein National Research Service award (T32 RR024394) as part of North Carolina State University's Comparative Medicine and Translational Research Training Program.

We thank Maria Stone, Stephen Stauffer, Mitsu Suyemoto, and the Laboratory for Advanced Electron and Light Optical Methods for their valuable technical assistance.

REFERENCES

1. **Aderka, D., J. M. Le, and J. Vilcek.** 1989. IL-6 inhibits lipopolysaccharide-induced tumor necrosis factor production in cultured human-monocytes, U937 cells, and in mice. J. Immunol. **143:**3517–3523.
2. **Agace, W., et al.** 1993. Selective cytokine production by epithelial cells following exposure to Escherichia coli. Infect. Immun. **61:**602–609.
3. **Bartges, J. W.** 2004. Diagnosis of urinary tract infections. Vet. Clin. North Am. Small Anim. Pract. **34:**923–933.
4. **Chin, A. C., W. Y. Lee, A. Nusrat, N. Vergnolle, and C. A. Parkos.** 2008. Neutrophil-mediated activation of epithelial protease-activated receptors-1 and -2 regulates barrier function and transepithelial migration. J. Immunol. **181:**5702–5710.
5. **Dinarello, C. A.** 2003. Interleukin=1 family [IL=1∓1, F2], p. 643–668. *In* A. W. Thompson and M. T. Lotze (ed.), The cytokine handbook, 4th ed., vol. 2. Academic Press, Boston, MA.
6. **Foxman, B., R. Barlow, H. D'Arcy, B. Gillespie, and J. D. Sobel.** 2000. Urinary tract infection: self-reported incidence and associated costs. Ann. Epidemiol. **10:**509–515.
7. **Foxman, B., and R. R. Frerichs.** 1985. Epidemiology of urinary-tract infection. 2. Diet, clothing, and urination habits. Am. J. Public Health **75:**1314–1317.
8. **Funfstuck, R., et al.** 2001. Secretion of cytokines by uroepithelial cells stimulated by Escherichia coli and Citrobacter spp. Int. J. Antimicrob. Agents **17:**253–258.
9. **Gabay, C.** 2006. Interleukin-6 and chronic inflammation. Arthritis Res. Ther. **8**(Suppl. 2)**:**S3.
10. **Gill, S. E., and W. C. Parks.** 2008. Metalloproteinases and their inhibitors: regulators of wound healing. Int. J. Biochem. Cell Biol. **40:**1334–1347.
11. **Gotte, M.** 2003. Syndecans in inflammation. FASEB J. **17:**575–591.
12. **Hang, L., B. Wullt, Z. X. Shen, D. Karpman, and C. Svanborg.** 1998. Cytokine repertoire of epithelial cells lining the human urinary tract. J. Urol. **159:**2185–2192.
13. **Hauser, P. J., et al.** 2009. Restoring barrier function to acid damaged bladder by intravesical chondroitin sulfate. J. Urol. **182:**2477–2482.
14. **Hedges, S., et al.** 1994. Uroepithelial cells are part of a mucosal cytokine network. Infect. Immun. **62:**2315–2321.
15. **Hedges, S., P. Anderson, G. Lidin-Janson, P. de Man, and C. Svanborg.** 1991. Interleukin-6 response to deliberate colonization of the human urinary tract with Gram-negative bacteria. Infect. Immun. **59:**421–427.
16. **Hedges, S., et al.** 1992. Comparison of urine and serum concentrations of interleukin-6 in women with acute pyelonephritis or asymptomatic bacteriuria. J. Infect. Dis. **166:**653–656.
17. **Hedges, S., M. Svensson, and C. Svanborg.** 1992. Interleukin-6 response of epithelial cell lines to bacterial stimulation in vitro. Infect. Immun. **60:**1295–1301.
18. **Hicks, R. M., B. Ketterer, and R. C. Warren.** 1974. Ultrastructure and chemistry of the luminal plasma membrane of mammalian urinary bladder: a structure with low permeability to water and ions. Philos. Trans. R. Soc. Lond. B Biol. Sci. **268:**23–38.
19. **Hiro, D., A. Ito, K. Matsuta, and Y. Mori.** 1986. Hyaluronic acid is an endogenous inducer of interleukin-1 production by human monocytes and rabbit macrophages. Biochem. Biophys. Res. Commun. **140:**715–722.
20. **Hooton, T. M., and W. E. Stamm.** 1997. Diagnosis and treatment of uncomplicated urinary tract infection. Infect. Dis. Clin. North Am. **11:**551–581.
21. **Hu, P., et al.** 2002. Role of membrane proteins in permeability barrier function: uroplakin ablation elevates urothelial permeability. Am. J. Physiol. Renal Physiol. **283:**F1200–F1207.
22. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection Escherichia coli isolate. Infect. Immun. **33:**933–938.
23. **Jantausch, B. A., R. O'Donnell, and B. L. Wiedermann.** 2000. Urinary interleukin-6 and interleukin-8 in children with urinary tract infection. Pediatr. Nephrol. **15:**236–240.
24. **Jones, M. R., et al.** 2006. Roles of interleukin-6 in activation of STAT proteins and recruitment of neutrophils during Escherichia coli pneumonia. J. Infect. Dis. **193:**360–369.
25. **Leemans, J. C., M. Vervoordeldonk, S. Florquin, K. P. van Kessel, and T. van der Poll.** 2002. Differential role of interleukin-6 in lung inflammation induced by lipoteichoic acid and peptidoglycan from Staphylococcus aureus. Am. J. Respir. Crit. Care Med. **165:**1445–1450.
26. **Lewis, S. A.** 2000. Everything you wanted to know about the bladder epithelium but were afraid to ask. Am. J. Physiol. Renal Physiol. **278:**F867–F874.
27. **Lewis, S. A., D. C. Eaton, and J. M. Diamond.** 1976. The mechanism of Na+ transport by rabbit urinary bladder. J. Membr. Biol. **28:**41–70.
28. **Mehrad, B., and T. J. Standiford.** 1999. Role of cytokines in pulmonary antimicrobial host defense. Immunol. Res. **20:**15–27.
29. **Naugler, W. E., and M. Karin.** 2008. The wolf in sheep's clothing: the role of interieukin-6 in immunity, inflammation and cancer. Trends Mol. Med. **14:**109–119.
30. **Negrete, H. O., J. P. Lavelle, J. Berg, S. A. Lewis, and M. L. Zeidel.** 1996. Permeability properties of the intact mammalian bladder epithelium. Am. J. Physiol. **271:**F886–F894.
31. **Ohsugi, Y.** 2007. Recent advances in immunopathophysiology of interleukin-6: an innovative therapeutic drug, tocilizumab (recombinant humanized anti-human interieukin-6 receptor antibody), unveils the mysterious etiology of immune-mediated inflammatory diseases. Biol. Pharm. Bull. **30:**2001–2006.
32. **Otto, G., J. Braconier, A. Andreasson, and C. Svanborg.** 1999. Interleukin-6 and disease severity in patients with bacteremic and nonbacteremic febrile urinary tract infection. J. Infect. Dis. **179:**172–179.
33. **Parsons, C. L.** 2007. The role of the urinary epithelium in the pathogenesis of interstitial cystitis/prostatitis/urethritis. Urology **69:**9–16.
34. **Parsons, C. L., D. Boychuk, S. Jones, R. Hurst, and H. Callahan.** 1990. Bladder surface glycosaminoglycans: an epithelial permeability barrier. J. Urol. **143:**139–142.
35. **Parsons, C. L., and S. G. Mulholland.** 1978. Bladder surface mucin—its antibacterial effect against various bacterial species. Am. J. Pathol. **93:**423–432.
36. **Parsons, C. L., C. W. Stauffer, and J. D. Schmidt.** 1988. Reversible inactivation of bladder surface glycosaminoglycan antibacterial activity by protamine sulfate. Infect. Immun. **56:**1341–1343.
37. **Quinton, L. J., et al.** 2008. Alveolar epithelial STAT3, IL-6 family cytokines, and host defense during Escherichia coli pneumonia. Am. J. Respir. Cell Mol. Biol. **38:**699–706.
38. **Rose-John, S., G. H. Waetzig, J. Scheller, J. Grotzinger, and D. Seegert.** 2007. The IL-6/sIL-6R complex as a novel target for therapeutic approaches. Expert Opin. Ther. Targets **11:**613–624.
39. **Schindler, R., et al.** 1990. Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. Blood **75:**40–47.
40. **Senior, D. F.** 2007. Management of urinary tract infections, p. 282–289. *In* J. Elliott and G. Grauer (ed.), BSAVA manual of canine and feline nephrology and urology, 2nd ed. British Small Animal Veterinary Association, Gloucester, United Kingdom.
41. **Svanborg, C., G. Godaly, and M. Hedlund.** 1999. Cytokine responses during mucosal infections: role in disease pathogenesis and host defence. Curr. Opin. Microbiol. **2:**99–105.
42. **Tilg, H., E. Trehu, M. B. Atkins, C. A. Dinarello, and J. W. Mier.** 1994. Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor P55. Blood **83:**113–118.
43. **Umegaki, H., et al.** 1996. Protective effect of interleukin-6 against the death of PC12 cells caused by serum deprivation or by the addition of a calcium ionophore. Biochem. Pharmacol. **52:**911–916.
44. **Wang, X. P., et al.** 2004. The interleukin-6 cytokine system regulates epidermal permeability barrier homeostasis. J. Invest. Dermatol. **123:**124–131.
45. **Wolters, P. J., et al.** 2009. Neutrophil-derived IL-6 limits alveolar barrier disruption in experimental ventilator-induced lung injury. J. Immunol. **182:**8056–8062.
46. **Xing, Z., et al.** 1998. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. J. Clin. Invest. **101:**311–320.

Editor: A. J. Bäumler