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Acute Bacterial Inflammation of the Mouse Prostate

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Abstract

BACKGROUND—Prostatic inflammation is gaining increasing attention as a potential etiologic factor in prostate cancer, benign prostatic hyperplasia, lower urinary tract symptoms, and CPPS. This study was performed to address the need for a well characterized model of acute prostatic inflammation that may be used to study the effect of acute inflammation on epithelial and stromal cell proliferation, voiding behavior, and neurovascular physiology.

METHODS—Uropathogenic *E. coli* 1677 was instilled transurethrally into adult C57BL/6J male mice. Prostates were analyzed at 1, 2, 3, 5, 7, or 14 days post-instillation and compared to saline-instilled and naïve controls. Time course and severity of inflammation were characterized by the quantity and type of inflammatory infiltrate present, hemorrhage, proliferation, and reactive hyperplasia. RT-PCR was performed to characterize inflammatory mediators including IL-1 α , IL-1 β , IL-1RA, IL-18, IL-6, IL-10, IL-8, TNF α , and COX-2.

RESULTS—Inflammation was evident in all lobes of the prostate with the DLP most severely affected. Infection consistently led to a significant increase in neutrophils and macrophages in the early stages of prostate infection, followed by lymphocytic inflammation at the later time points. Inflammation was accompanied by induction of several inflammatory genes, including IL-1 family members, IL-6, and COX-2, and induced a significant increase in epithelial proliferation and reactive hyperplasia in all three prostate lobes.

CONCLUSIONS—Transurethral inoculation of uropathogenic *E. coli* 1677 reliably infects the mouse prostate, produces a significant inflammatory response, and induces quantifiable epithelial proliferation and reactive hyperplasia.

Keywords

inflammation; model; hyperplasia; *E. coli*

INTRODUCTION

Prostatic inflammation is common in the adult male population. As defined by the presence of inflammatory cells within the prostate gland, chronic inflammation is present in 44–54% of prostate samples collected during autopsy from men with no indication of prostate disease [1,2]. The etiology of prostate inflammation is likely multi-factorial, involving infectious

agents, dietary influences, environmental exposures, and metabolic processes, among others [3]. Reflux of urine into the intra-prostatic ducts has been implicated as a potential route of access for uropathogenic bacteria with the most frequently isolated bacteria being *E. coli* [4–6]. The frequency of asymptomatic or minimally symptomatic bacterial colonization and infection of the prostate is unknown; however, studies have implicated this as a relatively frequent, largely under-diagnosed event [7,8].

A recent epidemiological study determined that the percentage of men displaying symptoms of prostatitis (all types included) was 8.2% [9], demonstrating that the majority of prostatic inflammation is asymptomatic. However, there is accumulating evidence to suggest that prostatic inflammation is a significant etiologic factor in both prostate cancer and benign prostatic hyperplasia (BPH). Histopathological studies of human prostatectomy specimens identified lesions characterized by proliferating epithelial cells and activated inflammatory cells (proliferative inflammatory atrophy, PIA) adjacent to areas of prostatic intraepithelial neoplasia (PIN) and prostate cancer [10]. On the basis of this and subsequent studies, chronic inflammation is now widely considered as both a critical element in the genesis of PIA and a likely precursor of PIN and prostate cancer [11–14]. In a prospective study of 167 prostates analyzed during autopsy examination, a significantly higher percentage of glands harboring BPH demonstrated chronic inflammation (75%) than glands without BPH (50%) [2]. Clinical trials have indicated a positive correlation of inflammation with symptomatic progression of BPH, risk for urinary retention and need for surgery [15,16]. This may explain the very high incidence of inflammation in surgical specimens of BPH [17]. The immunologic features of inflammation associated with BPH is characterized by an abundance of T cells and increased expression of a variety of inflammatory cytokines, including IL-1, IL-2, IL-4, IL-6, IL-7, IL-8, IL-13, IL-15, IL-17, IL-23, and IFN- γ [18,19].

Given the associations between inflammation and prostate disease, there is a need for well-characterized models of acute and chronic prostate inflammation in the prostate with which to study the inflammatory process and its effects on epithelial and stromal cell proliferation. Reliable experimental animal models of human diseases provide a unique opportunity to uncover disease-associated pathways, which are difficult to define in man. These models serve as resources for obtaining new research data and testing new therapeutic strategies. We present here a comprehensive characterization of the acute inflammatory response in a model of prostatitis in the mouse induced by transurethral inoculation of uropathogenic *E. coli* 1677.

MATERIALS AND METHODS

Transurethral Instillation

All procedures were performed on 8–12 week old C57BL/6J male mice (Jackson Laboratories) under the Institutional Animal Care and Use Committee guidelines of the University of Wisconsin-Madison. Mice were anesthetized with isoflurane and then catheterized using a lubricated sterile polyethylene catheter (intramedic PE-10) per urethra. Instillation into the bladder and prostate was then performed with either uropathogenic *E. coli* 1677 in DPBS (2×10^6 /ml, 200 μ l per mouse) or an equal volume of DPBS alone.

Un-instrumented age-equivalent (naïve) mice used to control for any effects of catheterization and/or instillation. Animals were then sacrificed at 1, 2, 3, 5, 7, or 14 days post-instillation. Mice were injected with BrdU intraperitoneal 1 hr prior to sacrifice and prostates were collected and separated by lobe (VP, CG, and DLP) for further analysis. One lobe from each prostate was bisected and utilized for bacterial counts, RNA isolation, and/or histology. Six to nine mice per time point and per treatment or control (both sham and naïve) were collected for PCR analysis. Three mice per time point and per treatment or control

(both sham and naïve) were analyzed for histology and proliferation. Four mice per time point and per treatment (infection or saline groups only) were collected for bacterial counts.

Bacterial Counts

Prostate lobes were weighed, homogenized in Delbecco's phosphate buffered saline, and serially diluted to 1:100 and 1:1,000. Standard plate counts were calculated by plating onto Levine's EMB agar (Becton, Dickinson, and Co.) at 37°C. Prostate bacterial titers are represented as the number of colony-forming units calculated per mg of prostate tissue (Table I).

H&E Staining and Immunohistochemistry

Tissues for H&E staining and IHC were fixed in 10% formalin, imbedded in paraffin and serially sectioned at 5 µm. Antigen retrieval was achieved by boiling in 10 mM citrate buffer solution (citrate buffer stock solution consisting of monohydrate-free acid citric acid, sodium citrate dihydrate, pH 6.0) for 15 min. Blocking for nonspecific background was performed using IHC Background Buster solution (Innovex Biosciences) for 30 min at room temperature. Primary antibodies included BrdU labeling Detection Kit II (1:20, Roche) and rabbit anti-p63 monoclonal antibody IgG (1:100 or 1:200, Santa Cruz). Slides were co-incubated at room temperature overnight with both primary antibodies. They were then incubated for 1 hr at room temperature with a mixture of the secondary antibodies [donkey anti-mouse IgG-Alexa 488 conjugated antibody (1:100, Invitrogen) and donkey anti-rabbit IgG-Alexa 594 conjugated antibody (1:100, Invitrogen)], followed by 10 min incubation with Hoechst 33258 nuclear stain (1 µg/ml). The slides were finished by applying mounting media and cover slips then imaged using an Olympus fluorescent microscope and digital camera using SPOT QE software.

Inflammatory Grading

The severity of the inflammatory response was graded in three random 20× fields of H&E sections from each lobe (AP, DLP, and VP) according to four criteria: inflammatory infiltrate, tissue damage, hyperplastic response, and hemorrhage. The three prostate lobes exhibited differing responses to inflammatory stimulus and therefore lobes were graded separately based on the criteria shown in Table II.

Inflammatory infiltrate—Leukocytes were counted and each field given a score as follows: 0, no leukocytes; 1 (mild) less than 10 leukocytes; 2 (moderate) 10–30 leukocytes; or 3 (severe) greater than 30 leukocytes.

Tissue damage—Each field scored for severity and focality of tissue damage based on the integrity of the epithelium and the presence of visibly damaged cells (or pyknotic nuclei). Severity was scored as follows: 0, full epithelial integrity and no damaged cells; 1 (mild) sloughed epithelium in less than 25% of a ductal cross section and/or 1–20 damaged cells; 2 (moderate) sloughed epithelium in greater than 25% of ductal cross section and/or more than 20 damaged cell; or 3 (severe) complete epithelial slough.

Hyperplasia—Hyperplasia was scored as follows: 0, normal pseudostratified epithelium; 1 (mild) basal cell expansion resulting in a stratified bilayer; 2 (moderate) thickened epithelium (3–5 cell layers); or 3 (severe) thickened epithelium (greater than 5 cell layers).

Hemorrhage—Each field was scored for hemorrhage based on the presence and number of extravascular erythrocytes: 0, no free erythrocytes; 1 (mild) less than 10 erythrocytes; 2 (moderate) 10–30 erythrocytes; or 3 (severe) greater than 30 erythrocytes.

*Focal*ity for each criterion was scored as follows: 0, no evidence of the criterion occurring; 1 (focal) criterion occurs in 1 location; 2 (limited) criterion occurs in less than 25% of the field; 3 (intermediate) criterion occurs in 25–50% of the field; 4 (widespread) criterion found in greater than 50% of the field. The severity and focality scores were multiplied together for each criterion and a single score per 20× field (e.g., severity of 3, focality of 4 = 12) and the average of three determinations per field were averaged per data point. For all four scores, sections from three animals were scored for each group (n = 3) and data are presented as the mean ± standard error of the mean (SEM).

Characterization of the inflammatory infiltrate—Because the DLP responded with the most pronounced inflammation, we chose this lobe for further characterization of the inflammatory infiltrate. The number and type of leukocytes were characterized in the DLP at 0, 1, 2, 3, 5, 7, and 14 days post-inoculation. Neutrophils were counted utilizing slides stained with H&E. T lymphocytes, B lymphocytes, and macrophages were counted utilizing IHC for cells surface markers. Primary antibodies included rabbit anti-CD3 (T lymphocytes) polyclonal antibody (1:100, Dako), mouse anti-CD20 (B lymphocytes) monoclonal antibody (1:100, BP Pharm), and rat anti-F4/80 (monocytes/macrophages) monoclonal antibody (1:50, eBioscience). Secondary antibodies included donkey anti-rabbit IgG-Alexa 594 conjugated antibody (Invitrogen), donkey anti-mouse IgG-Alexa 488 conjugated antibody (1:200, Invitrogen), and donkey anti-rat-IgG-Alexa 594 conjugated antibody (1:200, Invitrogen), respectively. The protocol for IHC is described previously [20]. Data are presented as the mean number of cells ± standard error of the mean per 20× field.

RNA Extraction and cDNA

Prostate lobe halves were snap frozen in liquid nitrogen immediately upon removal from the animal. RNA extraction was performed using RNeasy columns in conditions recommended by the manufacturer for tissue isolate (Qiagen, Inc.) and RNA yield was measured by 260/280 nm ratio on a spectrophotometer (Bio-Rad Labs) or Nanodrop 1000 (Thermo Scientific Inc.). cDNA was created by reverse transcription and was performed as previously described [21].

Semi-Quantitative Real-Time PCR

PCR analysis of cDNA was performed on all samples to obtain gene expression profiles using primers for the selected inflammatory mediators IL-1 α , IL-1 β , IL-1RA, IL-18, IL-6, IL-10, IL-8, TNF α , and COX-2 (Table III). Primers were created using the NCIB mouse DNA database and Primer-BLAST and the Primer-3 program. All primers were made at the Biotechnology Center-DNA synthesis laboratory (UW-Madison, WI). PCR reactions were run and products detected by SYBR green fluorescence (Applied Biosystems on real time-thermocyclers, iCycler or MyiQ (Bio-Rad Labs)). Expression levels were normalized to the housekeeping gene S27 and reported as a ratio of the gene of interest to S27 (fold change over naïve).

Statistical Analysis

Analysis was performed with the assistance of the Biostatistics Core of the Department of Surgery at the University of Wisconsin. Two-way analysis of variance (ANOVA) was performed to determine the significance of treatment group and day of sacrifice. For analysis of proliferation, an additional term was added to adjust for block effects. When appropriate (two-sided $P < 0.05$), pair-wise comparisons were obtained (Fisher's protected least squares differences [LSD]). Residual plots were used to assess model assumptions, and transformations of the dependent variable were pursued to ameliorate possible violations. Figures were designed in R 2.8.1 [22], and the ANOVA models were analyzed with Proc

GLM of SAS 9.1.3 (SAS Institute, Cary, NC). The following transformations were analyzed: square root for BrdU cell count and the natural logarithm of PCR expression data plus 0.1 [$\ln(y + 0.1)$].

RESULTS

Bacterial Inoculation and Prostatic Infection

Adult mice were catheterized per urethra and instilled with either uropathogenic *E. coli* 1677 in DPBS (2×10^6 /ml, 200 μ l per mouse) or an equal volume of DPBS alone. Four mice in each group were sacrificed at 3, 5, 7, and 14 days after inoculation and the prostate lobes processed for serial dilution and quantitative bacterial assay. No bacteria were present in saline instilled animals. Bacteria were generally present in all three prostate lobes following instillation of *E. coli* (Table I). Bacterial titers were highest during the first 5 days and dramatically decreased or gone by 7 days; two animals harbored low concentrations of bacteria up to 14 days after inoculation. Bacterial titers were consistently lower in the VP than either the CG or DLP. To confirm that instillation distributed bacteria to each prostate lobe, we performed mock instillations with 200 μ l blue carbon dye. Immediate sacrifice and examination revealed the presence of dye in all prostate lobes as well as the urinary bladder (data not shown).

Histological Grading

H&E stained slides were scored for severity of inflammation as described in the “Materials and Methods” Section using the criteria shown in Table II. A representative section of infected DLP illustrates the histological correlates of these criteria (Fig. 1). The severity of inflammation in the infected animals at each time-point post-instillation is compared to saline instilled controls at the same time-point and to naïve animals (Fig. 2). Naïve animals showed normal prostatic histology with minimal inflammatory infiltrate, hyperplasia, vascular changes, or tissue damage. Saline-instilled controls were not significantly different, although some of the tissues did exhibit a mild inflammatory infiltrate (not shown). Significant inflammation was observed in all lobes of the infected prostates. This was most severe between 2 and 5 days post-inoculation and had largely resolved by 7 days post-inoculation. In general, inflammation was more intense in the CG and DLP than in the VP, mirroring the higher bacterial titers in those lobes.

Proliferative Response and Hyperplasia

Epithelial proliferation was quantitated by BrdU incorporation 1, 2, 3, 5, 7, and 14 days post-instillation and compared to naïve controls. Labeling indices were determined by immunostaining for BrdU (Figs. 3 and 4). Significantly increased epithelial proliferation was observed in all of the lobes of the infected prostates at one or more time points (Fig. 4). Reactive hyperplasia that we attribute to inflammation-induced epithelial proliferation was evident by the appearance of a multi-layered epithelium. Increased epithelial proliferation and hyperplasia were most pronounced in the CG and DLP—paralleling the severity of inflammation in these lobes.

Inflammatory Cell Characterization

The composition of the inflammatory cell infiltrate was characterized in the DLP at each time point following bacterial inoculation (Fig. 5). We chose the DLP for this analysis because inflammatory score was highest and most consistent in this lobe. Analysis of H&E stained sections revealed an intense neutrophilic infiltrate peaking 2 days post-inoculation and diminishing rapidly after 3 days post-inoculation. Lymphocytes were the dominant feature of the inflammatory infiltrate after 3 days post-inoculation. The lymphocytic

population was composed primarily of CD3⁺ T lymphocytes and secondarily by CD20⁺ B cells. F4-80⁺ macrophages appeared early and were most abundant 2 days post-inoculation. A lymphocytic infiltrate with accompanying macrophages predominated between days 3 and 5 and exhibited progressive resolution at later time points. Small foci of lymphocytes persisted in some specimens up to 14 days post-inoculation, suggesting progression toward chronic inflammation.

Inflammatory Mediator Expression

To gauge whether our model produces a characteristic mediator response, we quantitated expression of inflammatory mediator mRNA by RT-PCR in the DLP of infected and saline controls 2, 5, and 14 days post-inoculation and in the DLP of naïve animals (Fig. 6). Robust increases in cytokine expression were observed following bacterial inoculation. Expression of IL-1 α , IL-1 β , IL-18, Cox-2, IL-1RA, and IL-10 were all significantly increased at one or more time points during the inflammatory response following bacterial inoculation. Apparently increased mRNA expression for IL-8, TNF, and IL-6 did not achieve statistical significance due to considerable experimental variation. Instillation of saline also induced expression of IL1 α and IL-18.

DISCUSSION

We have characterized the pathological and molecular features of acute bacterial infection of the mouse prostate. Although there have been several publications describing the use of *E. coli* to induce acute bacterial prostatitis in the mouse [23–26], none have comprehensively reviewed the time course and severity of inflammation, including the quantity and type of inflammatory infiltrate present, inflammatory cytokines, hemorrhage, epithelial proliferation, and reactive hyperplasia throughout the entire acute phase of infection. Because there is accumulating evidence to suggest that prostatic inflammation is a significant etiologic factor in both prostate cancer and benign prostatic hyperplasia (BPH), characterizing the inflammatory response associated with bacterial prostatitis is important in understanding the pathologically conducive microenvironment that may favor the initiation and progression of cancer and/or BPH. Acknowledging that prostatitis and prostatic neoplasia likely have a multitietiological nature, the study of several animal models with unique features may be necessary in gathering evidence that chronic prostate inflammation may play a role in the development of BPH and prostate cancer. In addition, this model may be used to study the effect of acute inflammation on epithelial and stromal cell proliferation, voiding behavior, and neurovascular physiology.

In the model presented here, the response to bacterial inoculation is characterized by a primarily neutrophilic infiltrate in the first and second day that evolves to a primarily lymphocytic infiltrate 3–5 days post-inoculation. Macrophages are prevalent to a lesser degree throughout both stages. The second stage (days 3–5) has a very similar leukocytic cellular distribution as that found in chronic human prostatic inflammation [27,28]. Our quantitative studies of the time course of cellular infiltrate were characterized by remarkably little experimental variation (error) suggesting that the time course of the inflammatory response is highly reproducible and consistent. Histological evidence of inflammation was accompanied by increased expression of several inflammatory genes including IL-1 family members, IL-6, and COX-2.

The DLP exhibited the highest bacterial titers, the most robust inflammatory response and the most intense increase in proliferation and hyperplasia. The inflammatory response observed in the ventral prostate was minimal, and the inflammatory response in the coagulating gland (anterior prostate) was intermediate. To examine the possibility that the anatomic arrangement of the prostatic ducts creates a preferential shunting of instilled

bacteria into the DLP, we instilled both Affigel bead instillation and colored dyes per urethra. Immediate sacrifice and examination revealed that 200 μ l of fluid distributed to all lobes of the prostate as well as the bladder (data not shown). This may suggest that the DLP is innately more susceptible and responsive than the other lobes of the rodent prostate. Importantly, no tracking dye was found in the ureters or kidney of these animals, indicating that reflux to the upper tract is not an artifact of this method and volume of instillation.

Low titers of bacteria persisted in the prostate in two animals throughout the 14-day experiment. This unexpected finding suggests that low-titer bacterial colonization of the prostate might be a regular if infrequent occurrence. This has been suggested to occur in the human prostate based on several observations. One is the presence of 16S ribosomal RNA in prostate tissue [29,30]. Another is the finding of culturable bacteria in prostate tissue from men with negative preoperative urine cultures [31].

An unexpected finding was that some cytokines were induced in saline-instilled controls as compared to naïve animals in the absence of a significant inflammatory infiltrate. This suggests that the instillation process itself induces a response. One possibility is trauma from catheterization or instillation; however, there was no histologic evidence of this. Another is that instillation distends the prostate duct, producing stretch-induced changes in expression of mechanically sensitive inflammatory genes. This has been previously shown in urinary bladder [32–35]. A volume of 200 μ l was used because in our preliminary studies, instilling only 20 μ l of dye did not adequately enter all lobes of the prostate. As the saline control samples were culture-negative, and a neutrophilic response was not observed, bacterial contamination is unlikely the cause of increased inflammatory cytokines. This suggests a mechanical induction of these genes.

We observed increased epithelial proliferation and reactive hyperplasia as a result of induced inflammation. Inflammatory mediators appear to play a critical role in the proliferative response of the epithelium to inflammation. IL-1 secreted by either inflammatory infiltrate or epithelium during inflammation promotes epithelial proliferation and hyperplasia of the prostate via growth factor induction [20,36]. Additional inflammatory mediators known to promote epithelial proliferation of the prostate include RANKL, lipid mediators including prostanoids, and the CXCL family of chemokines [37–40]. An elegant IL-8 transgenic mouse line demonstrates that IL-8 over-expression induces proliferation and reactive hyperplasia in the mouse prostate [41]. This model of inflammation may provide a vehicle that can be used to further explore the mechanisms linking inflammation and hyperplasia in the prostate.

CONCLUSIONS

Transurethral instillation of *E. coli* into adult male mice reliably produces infection of the mouse prostate, produces a significant inflammatory response, and induces quantifiable epithelial proliferation and reactive hyperplasia. Characteristics of this inflammatory response include a characteristic time course of leukocytic infiltration, induction of inflammatory cytokines, resolution of the inflammatory response and occasional persistence of low titer bacterial colonization.

Abbreviations

BPH	benign prostatic hyperplasia
BrdU	5-bromo-2-deoxyuridine
CaP	prostate cancer

CFU	colony forming unit
CG	coagulating gland
DLP	dorsal-lateral prostate
DPBS	Delbecco's phosphate buffered saline
EMB	eosin methylene blue
H&E	hematoxylin and eosin
IHC	immunohistochemistry
IL	interleukin
OE	over expression
PCR	polymerase chain reaction
PIA	proliferative inflammatory atrophy
PIN	prostatic intraepithelial neoplasia
RNA	ribonucleic acid
RT	real time
VP	ventral prostate

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Fig. 1. Representative H&E stained sections of the DLP 5 days after instillation of saline (top panels) or *E. coli* 1677 (bottom panels). The 10× power images are shown on the left; 40× power insets are shown on the right and specific features of inflammation are designated in the 40× image of the infected DLP: (H) indicates hyperplasia; (V) indicates vascular damage; (I) indicates inflammatory infiltrate; and (PN) indicates a pyknotic nucleus. The central lumen is filled with necrotic epithelium and inflammatory infiltrate.

Fig. 2.

Comparison of hyperplasia, inflammatory infiltrate, vascular pathology and tissue damage in the VP, CG, and DLP of infected and saline control prostates 1, 2, 3, 5, 7, and 14 days post instillation ($n = 3$ at all time points). Inflammation scores for naïve controls ($n = 3$) are shown at all time points for purpose of comparison. * $P < 0.05$ compared to naïve, and # $P < 0.05$ compared to saline by ANOVA.

Fig. 3. Immunostaining for BrdU (green) in the DLP (20×). Co-staining for p63 (red) was performed to identify the basal cell layer. **A:**Naïve control; **(B)** saline control 2 days post-instillation; **(C)** saline control 5 days post-instillation; **(D)** infected 2 days post-inoculation; **(E)** Infected 5days post-inoculation.

Fig. 4.

A–C: Comparison of epithelial proliferation rates determined as the number of BrdU positive cells per duct in the VP, CG, and DLP of infected and saline control prostates 1, 2, 3, 5, 7, and 14 days post instillation ($n = 3$ at all time points). Proliferation rates for naïve controls ($n = 3$) are shown at all time points for purpose of comparison. * $P < 0.05$ compared to naïve; # $P < 0.05$ compared to saline by ANOVA with a square root transformation.

Fig. 5.

A–D: Characterization of DLP inflammatory infiltrate at 1–14 days post-inoculation by H&E staining and IHC presented as the mean number of cells per 20× field. **A:**Neutrophils, **(B)** Blymphocytes, **(C)** Tlymphocytes, **(D)** monocytes/macrophages. * $P < 0.05$ [ANOVA] compared to Day 0.

Fig. 6. RNA expression of inflammatory mediators in the DLP at selected time points after bacterial inoculation (squares) of saline instillation (diamonds) expressed as fold change from naïve: (A) IL-1 α , (B) IL-1 β , (C) IL-18, (D) IL-8, (E) COX-2, (F) TNF α , (G) IL-1RA, (H) IL-10, (I) IL-6. * P < 0.05 compared to naïve; # P < 0.05 compared to saline by ANOVA with a natural logarithmic transformation (n = 5–9 per time point).

TABLE I

Prostate Tissue Bacterial Titer ($\times 10^3$) at Selected Time Points After Instillation of Saline or Inoculation With *E. coli* 1677

Time point post-instillation (day)	Saline ^a	VP ^a	CG ^a	DLP ^a
3	0	0.31 (± 0.25)	2.38 (± 1.22)	49.9 (± 33.6)
5	0	0.4 (± 0.21)	55.1 (± 34.4)	65.0 (± 44.3)
7	0	0.011 (± 0.011)	0.059 (± 0.29)	1.62 (± 0.96)
14	0	0.001 (± 0.001)	0.06 (± 0.04)	2.09 (± 1.56)

None of the saline samples showed any evidence of infection or contamination.

^a n = 4, averaged.

TABLE II

Criteria Used for Scoring Intensity and Focality of Inflammation

Intensity	Inflammatory infiltrate	Tissue damage	Hyperplasia	Vascular pathology
0	Absent	No infiltrating leukocytes	Pseudostratified bilayer	No extravascular erythrocytes
1	Mild	<10 Leukocytes/field	Two clear layers-basal or luminal	Hemorrhage <10 erythrocytes
2	Moderate	10–30 Leukocytes/field	Expansion 3–5 epithelial cell layers	Hemorrhage 10–30 extravascular erythrocytes
3	Severe	>30 Leukocytes/field	Expansion greater than 5 epithelial cell layers	Hemorrhage >30 extravascular erythrocytes
Focality				
0	Absent	No evidence of inflammatory criterion		
1	Focal	Found in 1 location		
2	Limited	Found in 1–25% of tissue, stromal		
3	Intermediate	Found in 25–50% of stromal or epithelial tissue		
4	Widespread	Found in >50% of stromal/epithelial tissue; glandular infiltrate present		

TABLE III

Primer Sequences (5'–3') Used for RT-PCR Analysis

Mediator	Forward sequence	Reverse sequence
IL-1 α	CCG ACC TCA TTT TCT TCT GG	GTG CAC CCG ACT TTG TTC TT
IL-1 β	CTG CTT CCA AAC CTT TGA CC	AGC TTC TCC ACA GCC ACA AT
IL-1RA	TGG CCT AGG TGT CTT CTG CT	TGG TCT GGA CTG TGG AAG TG
IL-6	AGT TGC CTT CTT GGG ACT GA	GAC AGG TCT GTT GGG AGT GG
IL-8 (KC)	GCT GGG ATT CAC CTC AAG AA	GAA GTG GCA GAA GCT AAC CG
IL-10	CAT GGG TCT TGG GAA GAG AA	AAC TGG CCA CAG TTT TCA GG
IL-18	ACA ACT TTG GCC GAC TTC AC	GGG TTC ACT GGC ACT TTG AT
COX-2	CCA GAT GCT ATC TTT GGG GA	GCT CGG CTT CCA GTA TTG AG
TNF α	AGC CCC CAG TCT GTA TCC TT	GAG GCA ACC TGA CCA CTC TC
S27	TCT TTA GCC ATG CAC AAA CG	TTT CAG TGC TGC TTC CTC CT