THE UNIVERSITY OF CALGARY

The Inflammatory Process in Rat Models of Prostatitis

by

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A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

Department of Biological Sciences

Calgary, Alberta

October, 2005

APPROVAL PAGE

UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the faculty of Graduate Studies for acceptance, a thesis entitled "The Inflammatory Process in Rat Models of Prostatitis" submitted by Michael D. Lang in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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29 September 2005

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ABSTRACT

The pathology of many diseases comes from the inflammatory reaction against pathogens or "foreign" molecules. Mucosal surfaces are a first line of defence against such insults, so they often experience inflammatory responses.

Researchers hope to create experimental systems to replicate what is observed in the live disease state. Isolated laboratory environments are beneficial, allowing control over contaminants and environmental influence. Unfortunately, *in vitro* strategies cannot be directly applied to humans because of the complexity of living beings.

One method to solve this problem uses animal models to test techniques, then possibly extrapolate the results to the human system. Here, I explore inflammation at a mucosal surface, specifically that of the prostate; the mucosal coating of the prostatic ducts are key components of prostatitis. A pre-existing model of bacterial prostatitis was employed to observe characteristics of the infection and inflammation. A model for abacterial prostatitis was created, to study, the outcomes of experimental manipulation *in vivo.*

ACKNOWLEDGEMENTS

There are many people to whom I owe a debt of gratitude. First and foremost is my supervisor, Dr. Howard Ceri. I cannot emphasize enough how I valued his leadership and support over the years. I also thank the other members of my graduate committee for their suggestions and assistance. The members of the Ceri lab have always been a great resource for ideas, techniques, and fun. On a more general note, I wish to thank the Departments of Graduate Studies and Biological Sciences. Their generosity, concern, and assistance has been amazing, and I am truly grateful. Of course I cannot forget to acknowledge my parents for all they've done for me. Lastly, I need to thank my darling wife, Crystal. Words cannot express my gratitude and love.

DEDICATION

For Crystal,

My soul-mate, my angel, my best friend. I dedicate this work to you – it would never have been completed if not for you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES AND GRAPHS

LIST OF SYMBOLS/ABBREVIATIONS/NOMENCLATURE

CFU	Colony Forming Units
CPPS	Chronic Prostatitis/Chronic Pelvic Pain Syndrome
CNF1	Cytotoxic Necrotizing Factor Type 1
DAB	Diamino Benzidine Tetrahydrochoride
DNBS	Dinitrobenzenesulfonic Acid
ELISA	Enzyme-linked Immunosorbent Assay
EPS	Expressed Prostatic Fluid
GAG	Glycosaminoglycan
GAR/HRP	Goat anti-rabbit with horseradish peroxidase
GRO-CINC	Growth-regulated oncogene/cytokine-induced neutrophil chemoattractant
HLA	Human Leukocyte Antigen
HTAB	Hexadecyltrimethyl Ammonium Bromide
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL-1β	Interleukin-1β
IL-1Ra	Interleukin-1 Receptor Antagonist
LTB_4	Leukotriene B_4
MHC II	Major Histocompatibility Complex II
MPO	Myeloperoxidase
NSAIDs	Non-steroidal Anti-inflammatory Drugs

PAP	Rabbit Anti-HRP
PMN	Polymorphonuclear Leukocytes
TNF-α	Tumor Necrosis Factor alpha
UPEC	Uropathogenic *E. coli*
UTI	Urinary Tract Infection

I. INTRODUCTION

A. Background

The use of live animal models is a very important aspect of biological and medical research. While *in vitro* experiments done in a laboratory can produce reliable and intriguing results, *in vivo* modeling with live organisms can provide insights unavailable in an isolated, *in vitro* system. Simulating human diseases provides the opportunity to investigate disease processes, causes, and possible treatments.

B. Prostatitis

The prostate gland is prone to many diseases. Prostatitis is an inflammatory disease of the prostate gland, with various etiologies. It has been estimated that nearly 50% of men will have some prostatitis symptoms during their lifetimes, and 25% of urogenital problems treated in American males are due to prostatitis (92).

Prostatitis is an all-encompassing term that should be broken down into categories. An official classification strategy has been outlined by the NIH (National Institute of Health) to encompass all of the manifestations of

prostatic inflammation in order to standardize the terminology and treatment strategies within this field of study. These classifications are outlined in Table 1 (adapted from Nickel, 1999) (65). Here, I will discuss only the true inflammatory conditions that lead to clinical presentation and symptoms: categories I, II, and III A.

1. Acute Bacterial Prostatitis (Category I)

a. Etiology and presentation

Ten to twenty percent of prostatitis cases are found to have a microbial origin; of these, roughly half present as acute prostatitis (50). Patients tend to experience dysuria, urination urgency and frequency, suprapubic pain, and often chills, nausea, and vomiting (51). Most cases are also associated with decreased urination ability and possible urine retention. *Escherichia coli* (*E. coli*) is the most common uropathogen isolated from prostatitis sufferers (20). Other gram-negative organisms such as *Klebsiella* and *Proteus* may also contribute, while gram-positive organisms are rare (63). It is believed that these pathogens originate from the urethra as an ascending urinary tract infection that establishes a biofilm within

Table 1. NIH prostatitis classification system.

Category I	Acute bacterial prostatitis
Category II	Chronic bacterial prostatitis
Category III Category IIIA Category IIIB	Chronic prostatitis/chronic pelvic pain syndrome Inflammatory (also called abacterial, nonbacterial, or idiopathic prostatitis) Non-inflammatory (also called prostatodynia)
Category IV	Asymptomatic inflammatory prostatitis

the prostatic acini (17, 67), possibly leading to a persistent chronic state.

Protection of the prostate relies mainly on a rapid phagocytic defense. The prostate contains resident leukocytes, including scattered lymphocytes, mast cells, and macrophages (21) but the immune system is not as highly developed as in other mucosal systems such as the gut or the lung (43). These resident leukocytes can respond to bacterial invasion through direct phagocytosis of invading bacteria (macrophages) and secretion of pro-inflammatory mediators (macrophages and lymphocytes). The prostatic epithelium itself has also been shown to contribute to this secretion, producing interleukin 1β (IL-1β), interleukin 3 (IL-3), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and nitric oxide (11, 87). These mediators can recruit and activate phagocytic cells, most importantly neutrophils. Neutrophils are the key inflammatory cells involved in acute bacterial prostatitis. Mild or early stages of infection result in scattered neutrophils within the prostatic stroma; these cells migrate to the ducts, and the ductal lumens are seen to have an intact epithelium with neutrophil infiltration (7). The neutrophil accumulation can progress, leading to glands totally filled with neutrophils, macrophages, and cell debris. Much of the debris is composed of dead neutrophils, but also includes sloughed epithelium that is shed as a result of the influx of leukocytes (7).

The humoral immune response is also involved in acute bacterial prostatitis. Assays of both serum and expressed prostatic fluid (EPS) have

suggested an antibody-mediated reaction to infection in the prostate. Case studies have shown increases in serum antigen-specific IgG and IgA, and marked elevation in local IgA responses (found in EPS) (46). These levels tend to sharply decline after treatment, then slowly recede to baseline after approximately 6 months (46).

Although prostatitis is a very common disease, the focus of study into its pathogenesis has primarily been clinical. Most literature regarding acute bacterial prostatitis involves case studies and treatment strategies, with little basic science exploring the nature of the inflammation. This lack of in-depth examination is most likely due to the ability to effectively treat acute bacterial prostatitis, preventing it from being a primary focus of research.

b. Treatment/resolution

Physical examination of the patient and laboratory evaluation of EPS are diagnostic for acute bacterial prostatitis. Elevated leukocyte counts and culturable microorganisms are indicative of this disease. Treatment is initiated upon diagnosis, and primarily consists of antibiotics, although catheter-assisted urinary drainage may be undertaken to release retained urine (62). Unfortunately, physical manipulation of the prostate during acute bacterial prostatitis is not favored, for fear of causing bacterial dissemination and sepsis (62). The antibiotics most often prescribed are tetracyclines and

fluoroquinolones (62). Treatment is very effective, yet incomplete clearance of bacteria may lead to chronic, episodic prostatic inflammation. This is a very important consideration, especially with the possibility of a biofilm mode of growth, leading to a more resistant bacterial colonization (64). Informed decisions regarding antibiotic choice and dose must therefore be made, requiring laboratory assessment of a patient's specific bacterial infection.

2. Chronic Bacterial Prostatitis (Category II)

a. Etiology and presentation

Those cases of bacterial prostatitis that persist after an initial onset and treatment are termed chronic bacterial prostatitis. As mentioned above, ineffective clearance of an acute infection may lead to a chronic state, but chronic bacterial prostatitis does not always require the previous presentation of an acute phase (7).

Unlike acute bacterial prostatitis, the chronic state is very subtle. Urgency, frequency, and pain are still evident, yet not as severe as in the acute form. The main difficulty is the chronicity, with recurring episodes of prostatitis often associated with other manifestations of urinary tract infection (82). As in acute bacterial prostatitis, the typical uropathogens involved are *E. coli, Klebsiella,* and other enteric organisms. These enter the prostate via

ascending urinary tract infection, lymphatic or hematogenous spread, or by repeated urine reflux into the prostate (82).

A rare granulomatous form of prostatitis may also occur, due to complications involved with bacillus Calmette-Guerin (BCG) immunotherapy of bladder carcinoma, or from infection by *Mycobacterium* or parasites (92). *Mycobacterium tuberculosis* may disseminate through the blood and establish an infection in the prostatic stroma. This results in a localized, self-limiting granuloma.

b. Inflammatory reaction

While neutrophils are key in acute bacterial prostatitis, chronic stages have a much greater influx of mononuclear cells. Mild inflammation exhibits periglandular leukocyte infiltration, primarily composed of lymphocytes and monocytes/macrophages. This may progress to a more severe reaction, resulting in glandular infiltration by macrophages and neutrophils, and marked periglandular mononuclear leukocytosis.

This inflammatory reaction is generally mild, and is histologically indistinguishable from category IV asymptomatic inflammatory prostatitis (92). It is unknown why similar inflammatory reactions can lead to symptoms in men with chronic bacterial prostatitis, while those with category IV prostatitis often display no symptoms. One may implicate the bacteria themselves as somehow contributing to the symptomology, yet this is unlikely. The

uropathogens involved in prostatitis are rarely invasive, and the inflammatory reaction is mainly localized to the infected ducts and surrounding stroma with no systemic dissemination. Adding to this argument is the occurrence of category IIIA, nonbacterial prostatitis. This disease involves no definite bacterial involvement, yet symptoms and histology are very similar to chronic bacterial prostatitis. The patient's genetics, environment, and individual inflammatory responses may therefore play a critical role in these diseases.

As with acute bacterial prostatitis, the inflammatory response in chronic bacterial prostatitis is poorly characterized. Beyond simple observations of the cells involved (see above), very little is known about the progression of this inflammation. The humoral response to chronic infections results in increased antigen-specific titres for IgA and IgG in the prostatic secretions, but unlike acute bacterial prostatitis the serum levels of antibody do not increase (46).

Granulomatous forms of prostatitis due to *M. tuberculosis* involve discrete, non-caseating granulomas with possible central necrosis (92). Mononuclear cells, epithelioid cells, and some neutrophils are generally present, and there is often a large population of eosinophils, yet no allergic component has been discovered.

c. Treatment/resolution

Chronic bacterial prostatitis is treated with antibiotics whenever episodes of symptoms arise. As in acute infection, fluoroquinolones are the primary choice due to their efficacy and penetration into the prostate (58). The main dilemma with chronic bacterial prostatitis is that it has proven very difficult to cure. Antibiotic therapy generally causes recession of symptoms, yet they frequently recur.

One theory is that the bacteria residing on the prostatic epithelium have established biofilms, a mode of growth known to be more resistant to antibiotics than their planktonic counterparts (20, 64, 66). Incomplete clearance of the infection may transiently decrease bacterial load, thereby decreasing the extent of the inflammatory reaction and subsequently alleviating symptoms. Persistent microorganisms will then have a refractory time while they reestablish a substantial infection. During this time a sub-clinical inflammatory response would be ongoing, leading to the subtle effects of chronic prostatitis. If the bacteria then regain a significant population size, a new episode of acute-like inflammation may be seen; this may be the basis behind the recurrent nature of chronic bacterial prostatitis.

Because of its recurrent nature and the difficulties in treating chronic bacterial prostatitis, it has been suggested that a vaccine may be effective (5). Elevated local IgA and IgG are seen, and boosting this response may help clear the infection through neutralization and phagocytosis. This technique has

proven somewhat successful in other forms of urogenital inflammation (9, 22), and may prove useful in the prostate.

3. Inflammatory Chronic Prostatitis/Chronic Pelvic Pain Syndrome (Category IIIA) and Non-Specific Granulomatous Prostatitis

a. Etiology and presentation

While the bacterial forms of prostatitis are well recognized, diagnosed, and have established treatments, they only make up one fifth of the inflammatory diseases of the prostate. Category IIIA, the nonbacterial, chronic inflammatory form of prostatitis totals 40% of the cases (50). This disease presents similar to chronic bacterial prostatitis, with men experiencing perineal pain, and urologic frequency, urgency, and dysuria (20). The unique identifier is an elevated leukocyte count in EPS coupled with a lack of culturable microorganisms. The current classification strategy suggests naming this disease chronic prostatitis/chronic pelvic pain syndrome, often referred to as CPPS or category IIIA prostatitis. For the remainder of this paper, the term CPPS will be used for category IIIA prostatitis.

Because of this lack of defined pathogen etiology, there have been many theories regarding what causes CPPS. One common hypothesis proposes CPPS is due to infection, involving organisms that are difficult to culture. *Chlamydia, Mycoplasma,* and fastidious gram-positive organisms have all been implicated

as possible responsible pathogens (20). These studies have involved various culture techniques, assaying serum antibody titres, and polymerase chain reaction (PCR) assays for prokaryotic DNA. Unfortunately, most of these studies have not been convincing due to lack of proper controls, basis on case studies with very small sample sizes, or directly conflicting results (19, 85).

A second hypothesis suggests that CPPS is an autoimmune disease. This argument is bolstered by findings that $CD4^+$ T cells from men with CPPS are reactive against seminal plasma (1), and more specifically to prostate specific antigen (PSA) (30). Animal models have replicated this situation, where prostate homogenate injected into healthy host animals (rats or mice) initiates a prostate-specific, autoimmune, T cell-mediated reaction that can be adoptively transferred to naïve animals (15, 18, 30).

A third hypothesis has been put forth, proposing that CPPS is due to chemically-induced inflammation resulting from urine reflux into the prostate (75). This study reveals a correlation between prostatitis symptoms and levels of urate and creatinine in the prostate. The authors suggest that these compounds may form crystals, leading to chronic inflammatory reactions. Complementary to this is our own experimental evidence that suggests a weakened mucosal barrier within the prostate may allow chemical mediators to incite inflammatory reactions (48).

One can envision a combinatorial etiology to this idiopathic disease. Initial insult (be it chemical or bacterial) may be enhanced by a loss of mucosal integrity due to altered secretion of mucosal components (71). This could lead to acute or subacute inflammation that can result in tissue damage and release of previously sequestered antigens. These autoantigens may initiate an autoimmune response. This antigenic release is not unprecedented, as acute bacterial prostatitis can lead to epithelial cell dissemination (23), and the severity of inflammation correlates with levels of circulating PSA in serum (59).

While not the same as CPPS, there is a granulomatous form of prostatitis that may also have an autoimmune component (80). It is rare, and as such will be considered here only briefly. Non-specific (idiopathic) granulomatous prostatitis is similar to the tuberculoid form, but no infectious organisms have been implicated (7). No etiology is defined, but it has been suggested that an obstruction of the ducts could lead to extravasation of secretions, thereby inciting a foreign-body inflammatory response (70). This reaction may also be triggered by surgery, as sites of transurethral resection have been shown to be associated with granulomatous prostatitis (10).

b. Inflammatory reaction

Histologically, CPPS is similar to chronic bacterial prostatitis. Neutrophils, macrophages, and lymphocytes primarily are seen, in stromal, periglandular,

and intraglandular locations. The cytology suggests a cell-mediated response, as there is an increase in antigen-presenting cells such as macrophages, and a marked predominance of $CD8^+$ T cells. There is also an increase in the expression of HLA-DR (MHC II) by prostatic epithelium in CPPS, suggesting it may play a cognate role in antigen presentation to T cells (7).

Examination of EPS in CPPS has revealed a significant increase in macrophages (3), IL-1β and TNF-α (60) compared to healthy individuals, and to those with non-inflammatory category IIIB prostatitis. This cytokine data has been confirmed by assaying seminal plasma directly (2), but has also been refuted, as one study has detailed no change in IL-1β between healthy and CPPS men (69).

On rare occasions, the inflammation may take a more granulomatous form. This too has been suggested to be autoimmune in origin, possibly a cell-mediated reaction to extravasated prostatic secretions that escape from the ducts into the stroma (16). The granulomas may be focal around the ducts, or disseminated throughout the prostatic tissue. Macrophages, T cells, and multinucleate giant cells are prevalent within these granulomas; neutrophils are fewer, and are seen primarily within the ducts (16).

c. Treatment/resolution

Currently, the primary treatment strategy for CPPS and non-specific granulomatous prostatitis is alleviation of symptoms. Repeated ejaculation or prostatic massage has been used to reduce fluid and cell load; heat treatment, transurethral ultrasound or megavoltage therapy alleviate pain. Anti-inflammatory drugs (NSAIDs) reduce the pain and inflammation (25), and α-blockers relax the bladder neck allowing increased micturition (93). The drawback to these strategies is that they are not cures, and as such must be repeated as long as the inflammation persists.

Attempts at cures have often focused an antibiotic therapy. Clinical studies have suggested that there may be a microbial cause, due to improvements seen in patients administered various antibiotics. Many of these arguments have since been refuted, due to improper controls, poor initial diagnosis, and placebo effect (25).

There are many theoretical strategies that may be applied for therapy of CPPS. These involve the use of allopurinol to remove urate crystals, cyclosporine to decrease autoimmunity, and cytokine manipulation to reduce inflammation (IL-1 receptor antagonist, thalidomide to inhibit TNF-α, or TGF-β to block cell-mediated immunity) (25). To date, these strategies are purely hypothetical or at best are being studied experimentally in animal models or in vitro. It is an exciting prospect that the inflammatory reaction and basic causative mechanisms are now being examined; finding a cure is now the drive, as opposed to emphasizing symptom alleviation.

C. Rationale and Hypothesis

Studying diseases has obvious medical value, and one of the best tools to do this is through the use of animal models. Because of a lack of previous models of abacterial prostatitis, our first goal was to develop such a model to facilitate investigation of the inflammatory reaction.

The mucosal systems of the human body are constantly bombarded by challenges from the outside environment. Fortunately, a complex protective mechanism resides along these sites; one important component of which is the epithelial layer. The epithelium comprises the outermost cell surface within mucosal tracts, and it is therefore the first to encounter bacterial pathogens. Pathogenic bacteria that enter a mucosal site will attempt to colonize, and possibly invade, the epithelial layer.

To induce an inflammatory response in test animals, the theory of a "mucosal barrier" was explored. This theory suggests that the mucosal layers that coat epithelial surfaces are key barriers that protect the tissue from potentially damaging external assault. In the case of abacterial prostatitis, it is my hypothesis that a compromised mucosal barrier can leave the underlying epithelium vulnerable to physical or chemical irritation. This may result in

prostatic inflammation with no pathogenic cause. An existing model for bacterial prostatitis was also used, allowing comparison of the two disease states.

The basis for this hypothesis stems from observations of human prostates with benign hyperplasia. Past research in our lab has suggested that there is an alteration of mucosal surface molecule expression, specifically affecting the lectin-mucin interactions, that occurs during benign hyperplasia (unpublished data). Normal tissue shows mucin and lectin co-distribution at the epithelial surface of the prostatic acini. Hyperplastic tissue shows a drastic reduction in these molecules - both lectins and mucins are absent from the epithelium. Furthermore, benign hyperplasia rates increase with age, suggesting a continual decline in mucosal integrity. This lack of mucosal protection in the prostatic acini could therefore account for the increased rates of inflammation seen to occur with age.

The link between inflammation and loss of mucosal surface ultrastructure is not unique to the prostate. This has been seen in other model systems such as those for colitis and interstitial cystitis. A reliable system for initiating colitis in animal models is the use of ethanol, a "barrier breaker", to strip away the mucosal barrier, and an irritant dissolved in the ethanol which then has access to the underlying tissue. Specifically, a model developed by Morris et al. (55) uses ethanol as a barrier breaker, and trinitrobenzenesulfonic acid (TNBS) as an irritant to initiate inflammation. A single application of this

mixture into the colon is sufficient to create both acute and chronic inflammatory responses. Interstitial cystitis, an inflammatory syndrome of the bladder, also has been replicated in animal models that involve corrupting the mucosal integrity. The carbohydrate and glycosaminoglycan (GAG) components of the mucus create a charge barrier in the bladder that prevents solutes from crossing the epithelial surface. By disrupting this charge barrier with protamine sulfate, Parsons et al. (72) have shown that an inflammatory reaction can be initiated in animal systems. Adding an irritant, such as urea or potassium, can exacerbate this effect (68).

D. Using Animal Models to Study Prostatitis

We consider that a rat model provides a good representation of the analogous disease process in humans because the rat prostate shares many morphological similarities with the human prostate and because the histology displayed in human prostatitis bears a very close resemblance to rat prostatitis. Additionally, the initiation of infection in the rat model is via an ascending route through the urethra, a path that is believed to be the major means by which men become infected.

1. Abacterial Prostatitis Model

A poorly functioning mucosal barrier may allow potentially harmful agents (like those present in refluxing urine) access to the underlying epithelium in the ducts of the prostate, resulting in cell damage and subsequent inflammation. As discussed previously, the importance of the loss of mucosal integrity has been demonstrated in other animal models of inflammatory disease. Models of colitis (12) and interstitial cystitis (13) have focused on disrupting the normal mucosal barrier in a target organ, then observing the inflammation that develops. To explore the role of mucosal integrity in prostatic inflammation, we have developed a model of abacterial prostatitis in rats using a "barrier breaker" to strip away the mucosal lining of the prostate in coordination with a chemical to irritate the epithelium. A combination of ethanol and dinitrobenzenesulfonic acid (DNBS) has proven to be effective in inciting inflammation in animal models of colitis (12). We have adapted this model, in combination with techniques from a model of bacterial prostatitis (26), to study abacterial prostatitis.

With this model we assessed inflammation by assaying levels of the proinflammatory cytokines interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α).

2. Bacterial Prostatitis Models (CNF Models)

Bacterial prostatitis, characterized by symptoms of urinary tract infection (UTI), accounts for 5-10% of all prostatitis cases. The primary etiological

agent of bacterial prostatitis is *Escherichia coli* (20), and many of the disease-causing *E. coli* isolates express one or more virulence factors that include adhesive factors and toxins such as P fimbriae, cytotoxic necrotizing factor type 1 (CNF1), and hemolysin (51,60). The virulence capabilities of uropathogenic *E. coli* (UPEC) have been the focus of much research.

a. CNF Model 1

Epidemiological studies have linked CNF1 with *E. coli* strains that cause prostatitis, as well as uncomplicated UTI in women. Specifically, Mitsumori et al. reported that 64% of prostatitis isolates were cnf_1^+ (53), and Andreu and colleagues found that the percentages of cnf_1^+ prostatitis, pyelonephritis, and cystitis isolates were 63%, 48%, and 44%, respectively (4). In accordance with Andreu's findings, Terai and colleagues noted that 44% of *E. coli* isolates from ascending urethral bacterial prostatitis were cnf_1^+ (89). Isolates producing CNF1 have been collected from up to greater than 60% of men suffering from bacterial prostatitis. These statistics has prompted us to study the importance of CNF1 in acute bacterial prostatitis.

CNF1 is a chromosomally encoded UPEC toxin that catalyzes the deamidation of the small GTPases RhoA, Rac, and Cdc42 (29, 31, 49, 83). Deamidation of the GTPases renders these proteins constitutively active which, in most cells, leads to formation of actin stress fibers, lamellipodia, and filopodia. HEp-2 cells, which have been used as the prototypic cell for

evaluation of CNF1 toxicity, not only display actin stress fibers but also become multinucleated (13). Moreover, CNF1 has been reported to mediate a spectrum of additional phenotypic effects on cultured cells that include enhancement of phagocytosis in epithelial cells (26, 28) and reduction of CR3 receptor-dependent phagocytosis in monocytes (12, 26, 28). CNF1 also inhibits wound repair in T24 bladder cells and Hs 738 fibroblast cells (41), kills 5637 bladder cells through an apoptotic mechanism (52), effaces the brush border of T84 cells, and decreases the degree to which polymorphonuclear leukocytes migrate across a monolayer of those intestinal cells (37). Thus, CNF1 affects a variety of cellular functions *in vitro*, presumably through activation of the Rho GTPases.

Previous data suggests CNF1 is important in contributing to the severity of the inflammation in animal models of cystitis (79). Comparisons of the severity of disease caused by uropathogenic CNF1-positive *E. coli* strains and their isogenic CNF1-negative mutants have been completed. The production of CNF1 by an infecting UPEC strain evoked a more intense inflammatory response in the bladders of the animals when compared to bladders from animals inoculated with the isogenic CNF1-negative mutant even when bacterial counts were similar. Culture experiments with a CNF1-postive strain and its CNF1-negative isogenic mutant showed that human polymorphonuclear leukocytes (PMNs) kill CNF1-negative mutants more efficiently than they do the cognate CNF1-postive UPEC strain (81).

While these models imply CNF1 contributes to inflammatory damage in the urinary tract, its specific effects have yet to be determined *in vivo*. Here we expand upon the previously described acute bacterial prostatitis model (27) to more fully elucidate the effects of CNF1 on the inflammation associated with acute bacterial prostatitis. We looked at colonization and inflammatory damage in the rat prostate.

b. CNF Model 2

We compared the effects of a CNF-1 producing strain with those of its CNF1-negative isogenic mutant in a rat model of acute bacterial prostatitis (48, 66). To expand the understanding of the inflammatory process in this model, molecular events were the key focus. Specifically, cytokine levels in the prostate were measured after infection with one of the strains above.

3. IL-1Ra Models

a. Abacterial IL-1Ra Model

IL-1β is a key proinflammatory mediator involved in the inflammatory process. It is one of the first cytokines released in an inflammatory event, and has a varied array of cellular targets. IL-1β is secreted by many cell types, including epithelial cells and leukocytes.

We explored the importance of IL-1β in the above model of abacterial prostatitis by administering IL-1 receptor antagonist (IL-1Ra), a well-characterized inhibitor of IL-1β action. Because of its ability to bind to IL-1β receptors, the receptor antagonist blocks the binding of true IL-1β.

b. Bacterial IL-1Ra Model

To study the inflammation and the role of the inflammatory mediators, one can combine methods from the bacterial prostatitis model and the IL-1Ra study. Administration of IL-1Ra to rats with acute bacterial prostatitis will allow comparison to the abacterial IL-1Ra model.

II. METHODS AND MATERIALS

A. Barrier Breakers

The initial studies for modeling prostatitis must involve developing an animal model for abacterial prostatitis. In keeping with the theory that mucosal integrity is a key issue in prostate inflammation, we used the idea of "barrier breakers", as described for colitis research (91).

To interfere with endogenous lectins, in order to simulate mucosal component disruption, lactose (0.3 M) mixed with 150 mg TNBS in saline (0.1 mL) was instilled through a catheter. 150 mg DNBS in 50% ethanol (0.1 mL) was also tried. Three negative control were used; one had 50 mg/ml DNBS in phosphate buffered saline (PBS) (0.1 mL), a second had sucrose (0.3 M) in 150 mg TNBS in saline (0.1 mL), and a third had only 50% ethanol (0.1 mL). The gross and histological outcomes were compared to a previously described bacterial prostatitis model. We employed the model of bacterial prostatitis developed by Nickel et al. (66) (Figure 1) with modifications for the development of abacterial prostatitis. Male Sprague-Dawley (SD) rats of approximately 300 g were obtained from the University of Calgary Life and Environmental Sciences outbred colony. These were caged in polycarbonate shoe-box cages with corn cob bedding, and supplied with standard rat chow and tap water *ad libitum*. Rats were housed at 20 ± 2°C and 30 ± 10% humidity, with 12 h of illumination per day. Studies were done in accordance with animal care protocol of the University of Calgary.

B. Evans Blue Permeability

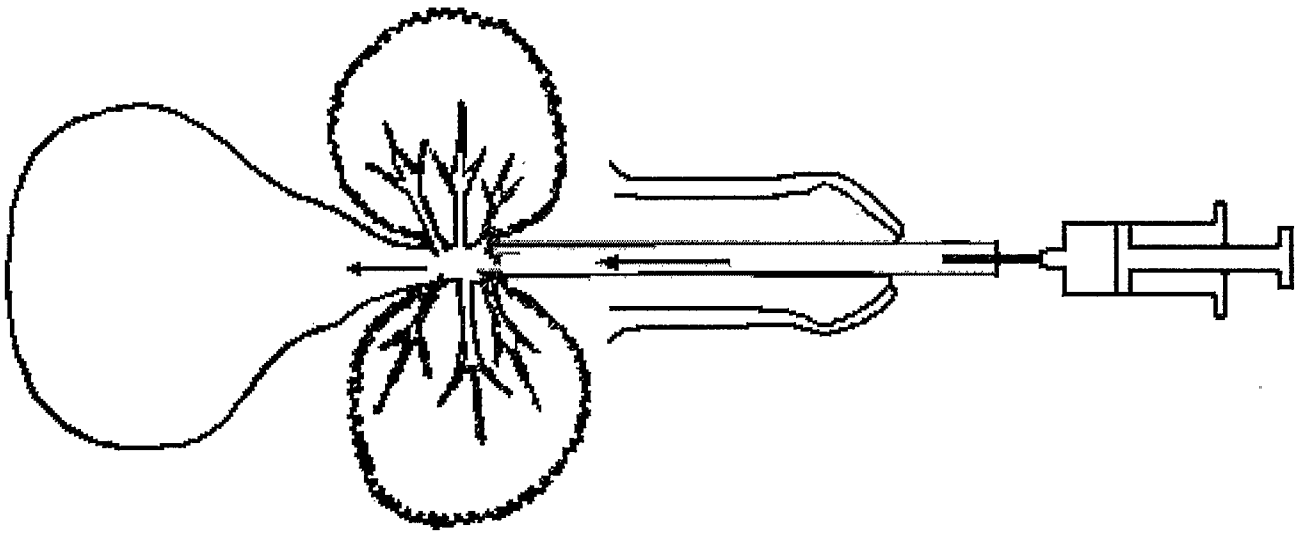
While intriguing, the results obtained from the lactose administration model were inconsistent, leading to the search for a more reliable barrier-breaker. To support ethanol as a viable barrier-breaker for modeling abacterial prostatitis, an *in vivo* procedure was devised using rats. Male SD rats (300 g)

were injected intramuscularly with 0.2 mL ketamine/xylazine, then their lower abdomens were shaved with clippers and sterilized with ethanol or betadine. 1 to 2 cm incisions were made to exteriorize the bladder, and small hemostats were used to occlude the bladder neck above the prostate.

Rats were catheterized to the prostate with lubricated, sterile PE 10 tubing on a 30 gauge needle. 0.1 mL of solution was instilled in each rat: CONTROL = 10% Evans blue (w/v) in sterile saline, TEST = 10% Evans blue (w/v) in 50% ethanol. After the catheters were withdrawn, the bladders were returned to the abdomens, and the animals were allowed to rest for 1 hour.

The rats were then euthanized and exsanguinated by cardiac puncture. Blood was collected in plasma separator tubes and centrifuged. The isolated plasma was then read on a spectrophotometer for absorbance at 620 nm (A620). These values were then compared between the test and control groups.

Figure 1. Schematic diagram of the catheter-mediated inoculation. Note the lobes of the prostate extending out from the urethra.



C. Abacterial Prostatitis Model

We employed the model of bacterial prostatitis developed by Nickel et al. (66) with modifications for the development of abacterial prostatitis. Male Sprague-Dawley rats of approximately 300 g were obtained from the University of Calgary Life and Environmental Sciences outbred colony. Studies were done in accordance with animal care protocol of the University of Calgary.

A test solution (designated T) was prepared by dissolving 100 mg/ml of DNBS in 50% ethanol in PBS. Two control sets were used, one to control for the effect of ethanol (an ethanol control containing 50% ethanol in PBS, designated EC), and the other to control for the effect of DNBS alone (a DNBS control containing 100 mg/ml DNBS in PBS, designated DC). Rats were anesthetized with 4% halothane, and catheterized with a polyethylene (PE 10) tube which had been sterilized and lubricated. 0.2 ml of test or control solution (EC or DC) was instilled, via the catheter, into the rat prostate at Time 0. The size and positioning of the catheter to allow instillation of solutions into the rat ventral prostate had previously been determined through the use of an opaque dye (66).

Ethanol and DNBS controls (n = 3) and test (n = 5) animals were sacrificed at times of 6, 12, 24, 48, and 168 hours by CO_2 asphyxiation.

Ventral prostates were removed and scored for gross morphology and histology. Three untreated rat prostates were collected as negative controls (designated UC for untreated control), and 2 rats with induced bacterial (*E. coli*) prostatitis were used as positive controls (designated BC for bacterial control); these were scored and processed. The bacterial prostatitis control followed exactly the model as outlined in Nickel et al. (66), with rats sacrificed after 7 days.

1. Gross Morphology Assay

An impartial observer blinded to the experiment scored the gross morphology of the prostates. Each was scored for edema, congestion, and hyperemia on a scale of 0 (no change from normal) to 3 (most severe). These were totaled for a combined gross morphological score from 0 to 9 for each prostate.

2. Histological Assay

Following the gross observations, the prostates were divided; one half was fixed in 10% neutral buffered formalin, the other processed for ELISA (enzyme-linked immunosorbent assay) (see below). The formalin-fixed tissues were embedded in paraffin for sectioning and staining with standard hematoxylin and eosin (H&E) protocols. Sections were scored, by an observer blinded to the experiment, for edema, leukocyte infiltration, and hemorrhage.

These criteria were each assigned a value of 0 (no change from normal) to 3 (most severe). These were totaled to obtain a final histological score (from 0 to 9) for each prostate.

a. Paraffin Embedding

Fixed tissues were placed in labeled cassettes and subjected to a standard series of ethanol and Americlear (a clearing solvent) baths. The ethanol baths dehydrate the tissues and fix the proteins by precipitation; the Americlear then permeabilizes the tissue so the paraffin can penetrate. Once the tissues were infused with the paraffin, they were embedded in wax blocks for sectioning.

b. Sectioning

The paraffin blocks containing the tissue samples were trimmed of excess wax, and thin sections were obtained for histological examination. A standard microtome was used to produce sections of 4-5 microns thickness. Sections were then collected on silane-coated microscope slides and allowed to dry overnight.

c. H&E Staining

After they were allowed to dry overnight, the slides were treated with a standard Hematoxylin/Eosin cellular stain. The slides underwent a series of solvent washes (Americlear, ethanol, Ehrlich's Alum Hematoxylin, and Eosin) to remove the paraffin, fix the tissues, and stain the cells.

3. Cytokine ELISA

The prostate tissue not fixed in formalin was homogenized in PBS containing 1 µg/ml each of leupeptin, pepstatin-A, and aprotinin (33). The homogenates were then centrifuged at 1800 g for 10 min and the supernatants were removed and frozen at -80°C until used. IL-1β and TNF-α ELISA (QuantikineM immunoassay, R&D Systems, Minneapolis, MN) were performed on these supernatants as per manufacturer's instructions.

4. Statistics

All statistics were calculated using Instat software (GraphPad Software, 1994). Sample sizes were determined to provide the lowest significant n value. Mann-Whitney U tests were used to compare the gross, histological, and ELISA results between control and test animals within each time group. A P value of less than 0.05 was considered significant.

D. Abacterial Prostatitis Early Time Points

Because of the lack of inflammatory characteristics early in the model, it was hypothesized that the timing window was too great between treatment and first sampling. It is possible that inflammatory markers were present early, peaked, and receded well before the first time-point was sampled.

To test this theory, the same abacterial prostatitis model was used, with all animals receiving ethanol and DNBS. Three rats were sacrificed at each of four time points post-catheterization. These were done at 0.5 hours, 1 hour, 2 hours, and 4 hours. Cytokine ELISAs were performed on prostate tissue of the animals, assaying for IL-1β and TNF-α. Any significant cytokine levels would indicate the original model missed a crucial time-point.

E. Abacterial Prostatitis 48 Hour Time-Point

Upon examination of the above initial studies, a 48-hour time-point was chosen as the focus of more extensive study. The above model was repeated, with ethanol and DNBS control (n=7) and test (n=15) animals sacrificed after 48 hours. Gross morphology, histology, and IL-1β assays were again performed as outlined.

F. Serum IL-1β Levels

To determine if the increase in cytokine levels in the prostate tissue in the abacterial prostatitis model is an isolated phenomenon, not systemic, blood from test and control rats was collected and the serum was removed. The IL-1β levels were assayed with an ELISA kit, as used in the previous models.

I. Bacterial Prostatitis CNF Model 1

1. Colonization

Groups of six male rats were anesthetized with 4% halothane, catheterized and infected with 2 x 10^5 CFU UPEC strain CP9 (an O4:H5:K54 isolate from the blood of a patient with pyelonephritis) or its CNF1-negative isogenic mutant CP9*cnf*$_1$ via the urethral catheter. 48 hours later, the rats were euthanized by CO_2 asphyxiation and their prostate glands were removed for analysis. The experiment was repeated three times with different doses of CP9 or CP9*cnf*$_1$.

Next, we performed a mixed infection experiment with a Lac-negative, CNF1-positive derivatives of CP9 (CP9*lacZ*) and CP9*cnf*$_1$ (Lac-positive, CNF1-negative), and C85 (O2:H, hemolysin-positive, CNF1-positive cystitis isolate) and C85*cnf*$_1$ (Lac-positive, CNF1-negative), to conclusively demonstrate the growth advantage of the CNF1-postive strains (79). Here five rats were inoculated with a mixture that contained 2 x 10^7 CFU of each strain and

euthanized two days later. Prostates were removed, homogenized, and serially diluted and plated for colony counts.

2. Gross morphology

Prior to homogenization for colony counts, the gross morphologies of all infected prostates (n=6) were scored. Prostates were observed for overt signs of inflammation, including edema, congestion and hyperemia. and given a score of 0 to 3 for each of these conditions (0 = none; 3 = the maximum score) and the three scores were totaled for a final score of 0 to 9. Examination of the gross morphologies of the infected prostates as well as the gross pathology score revealed differences between the CNF1-positive and CNF1-negative strains.

3. Histology

One half of each prostate was fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with H&E. Stained sections were observed microscopically and scored for histological signs of inflammation: edema, hemorrhage and leukocyte infiltration. Histological changes were scored in the same manner as for the gross morphological changes, *i.e.*, each of the three conditions was given a score of 0 to 3. Total scores of less than 2 were classified as mild inflammation, 2 to 4 were considered moderate

inflammation and scores of 5 or greater were categorized as severe inflammation.

J. Bacterial Prostatitis CNF Model 2

The previously described model of acute bacterial prostatitis was used (66, 67, 78), with some modifications. Male SD rats were divided into two groups (n = 5 per group), one group challenged with *E. coli* CP9 (CNF1-positive) and the other with its isogenic mutant, *E. coli* CP9*cnf*$_1$ (CNF1-negative). Animals were anesthetized with 4% halothane, and then catheterized with sterile polyethylene tubing. 0.2 ml of a 1×10^8 CFU/ml suspension of bacteria was instilled into the urethra. Rats were sacrificed 48 hours after catheterization and prostates were collected.

1. Colonization

Prostate homogenates were plated after serial dilution for enumeration of bacterial colonization.

2. Gross morphology

Gross morphological scoring of edema, hyperemia and congestion was completed, after which the prostates were sectioned and prepared for colony

counts, routine histology, myeloperoxidase (MPO) assay, and cytokine assays by ELISA as previously described (8, 48, 78). Each of the three categories comprising the overall gross score was given a value of 0 to 3. The three values were totaled (from 0 to 9) resulting in the final gross score.

3. Histology

Histological scoring of paraffin embedded sections was expanded upon from the previous studies described (48, 78). In addition to a general histological score, comprised of edema, hemorrhage, and leukocyte infiltration, a leukocyte score was also assigned. This involved observing interstitial leukocyte infiltrate, ductal leukocyte infiltration, and the extent of duct destruction. Each of the three categories comprising the histological and leukocyte scores was given a value of 0 to 3. The three values were totaled (from 0 to 9) resulting in the final histological and leukocyte scores.

4. Myeloperoxidase

The extent of neutrophil infiltration was estimated through the use of a MPO assay. Myeloperoxidase (MPO) is an enzyme found in the intracellular granules of neutrophils. The enzyme is therefore a marker of neutrophil content. Prostate tissue was homogenized in hexadecyltrimethyl ammonium bromide (HTAB) buffer then sonicated. After centrifugation, supernatants were mixed with O-dianisidine in phosphate buffer and assayed by kinetic

spectrophotometry at 450 nm. MPO levels from the two treatment groups were then compared.

5. Inflammatory Cytokines

To study the effects of CNF1 on inflammatory signaling, prostate homogenates were assayed for various inflammatory cytokines. IL-1β, growth-regulated oncogene/cytokine-induced neutrophil chemoattractant (GRO/CINC-1), and leukotriene B_4 (LTB_4) were examined. All assays were performed as per manufacturers' directions.

Data was compared between the two groups, and was analyzed statistically. One-way analysis of variance and Fisher's exact tests were used where appropriate, and a P value of 0.05 was considered significant.

G. Abacterial Prostatitis IL-1Ra Model

1. Interleukin-1 Receptor Antagonist

One common technique to inhibit IL-1β activity is the administration of interleukin-1 receptor antagonist (IL-1Ra). IL-1Ra has been used to decrease the inflammatory effects in a model of colitis (14). IL-1Ra has been shown to diminish inflammation in models of hippocampal necrosis (39), aortic

aneurysms (36), and sepsis (47). We carried out similar studies in our model of abacterial prostatitis in rats, to determine if IL-1Ra can diminish the inflammatory effects.

The experimental design was a modification of the original abacterial prostatitis model. Three groups of rats were used - 5 rats received, via urethral catheter, 0.2 ml of 100 mg/ml DNBS dissolved in 50% ethanol, with no IL-1Ra. These were a positive control for inflammation. 5 rats received 0.2 ml sterile PBS, these rats were injected with IL-1Ra. They served as a negative control for inflammation. The final group consisted of 10 rats receiving 0.2 ml of 100 mg/ml DNBS dissolved in 50% ethanol; they were also injected with IL-1Ra. These rats were the test animals to show the effects of IL-1Ra on inflammation.

The IL-1Ra injections combined strategies of Cominelli (14), Imai (39), and Hingorani (36). Each injection consisted of 0.5 ml sterile PBS containing 5 mg/kg purified IL-1Ra. These were given intraperitoneally 20 minutes before catheterization then 1, 9, 17, 25, 33, and 41 hours thereafter.

48 hours after the catheterization, all rats were sacrificed by CO_2 asphyxiation then their ventral prostates were removed. Gross morphology was scored by an impartial observer, grading edema, hyperemia, and congestion. The tissues were then processed for histology and MPO assay. The histological inflammation was scored for edema, hemorrhage, and leukocyte

infiltration. The data from all assays was compared among the three treatment groups to determine the effects of IL-1Ra on the inflammation.

2. ELISAs

IL-1β, LTB_4, and GRO-CINC ELISAs were performed. All ELISAs were carried out with pre-made kits, as per the manufacturer's instructions.

3. Myeloperoxidase Assay

Tissue was collected from control and test animals, and placed in scintillation vials. The samples were weighed and 100 ul HTAB buffer was added for every 50 mg tissue (2 ul/mg). Samples were then homogenized; the homogenates were then aliquotted into microfuge tubes and snap-frozen in liquid nitrogen. These samples were processed for the MPO assay as previously described.

H. Bacterial Prostatitis IL-1Ra Model

This study undertaken with IL 1Ra is similar to the previous work, but it involves bacterial prostatitis. By instilling uropathogenic *E. coli* CP9 into the prostate of male rats we can induce bacterial prostatitis. Inflammatory damage is seen, and levels of IL-1β are markedly higher than in healthy

animals. We used this model to explore the importance of IL-1β. Male rats were inoculated with bacteria, and were randomized into two groups. One group received IL-1Ra while the other received saline. IL-1Ra was injected intraperitoneally half an hour before bacterial inoculation, 1 hour after, then every 8 hours for 48 hours. The rats were then sacrificed and the prostates were assayed for signs of inflammatory damage. Gross morphology and various proinflammatory mediators (IL-1β, MPO) were measured.

III. RESULTS

A. Barrier Breakers

The administration of lactose into the prostates of male rats appeared to have a minor impact on the protective nature of the prostate ductal mucosa. While signs of inflammation in lactose/TNBS test rats were not drastic, edema and tissue congestion were apparent. Control prostates were a healthy color and consistency. The test group for DNBS/ethanol had signs of inflammation such as redness and edema (not shown). Histology showed open, healthy ducts in DNBS/PBS and ethanol alone control groups. DNBS/ethanol showed a dramatic difference. Leukocyte invasion into ducts and connective tissue were observed. This was not seen to near this extent in the lactose/TNBS test animals (not shown).

B. Evans Blue Permeability

To support ethanol as a viable barrier-breaker for modeling abacterial prostatitis, a straightforward test was devised. After treatment with ethanol or saline, male rats were catheterized with Evans Blue solution. Any color change detected in the animals' circulation was then due to the entrance of the dye through the prostate mucosa. As seen in Figure 2 the test animals, exposed to ethanol and Evans Blue showed increased absorbance values, indicating more dye was able to cross into their circulation through
the ductal epithelium within the prostates (untreated = 0.202 +/- 0.0144, saline = 0.175 +/- 0.0555, ethanol = 0.734 +/- 0.0613).

C. Abacterial Prostatitis Model

1. Gross Morphology Assay

The test prostates generally displayed signs of inflammation that were rare or not present in the control tissues. The final gross morphology scores are summarized in Figure 3. The 12 hour, 24 hour, and 48 hour time groups all showed significance between the test animals and those given DNBS in PBS (DC); $P < 0.05$. the n value for the 168 hour time group dropped below

statistically relevant levels. Three of the 5 test animals suffered severe prostatic inflammation and prostatic and urethral occlusion resulting in acute urinary retention and death.

2. Histological Examination

The scores for all time groups and controls are summarized in Figure 4. Test groups showed histological inflammation more pronounced than EC and DC, achieving statistical significance at 48 hours ($P < 0.05$), while the 6 hour and 12 hour groups only showed significance from their corresponding DC groups ($P < 0.05$). It should be noted that for the 24 hour group near significance ($P = 0.0714$) was reached; each 24 hour control group contained one animal with histological scores sufficient enough to raise the group average and prevent significance (data not shown).

3. Cytokine Levels

Figure 5 summarizes the ELISA data for the IL-1β assay. Significant increases in IL-1β levels were seen in the test rats compared to EC rats at 6 hours and 24 hours ($P < 0.05$); significant increases were also seen in test rats compared to DC rats at 12 hours, 24 hours, and 48 hours ($P < 0.05$). The untreated controls (UC) displayed minimal IL-1β levels (Fig.5), while the bacterial prostatitis controls (BC) showed levels much higher than any of the test animals, an average of 3752 pg/ml (data not shown).

The data for the TNF-α ELISA are seen in Figure 6. No significant differences were seen in the levels of this cytokine between treatment groups, but bacterial prostatitis rats displayed higher levels than all other animals.

D. Abacterial Prostatitis Early Time Points

Test animals sacrificed after 4 different early time-points revealed no drastic increase in IL-1β or TNF-α (Fig. 7).

E. Abacterial Prostatitis 48 Hour Time Point

Based on the previous findings of the abacterial model, the 48 hour time point was expanded upon, using larger sample sizes. As can be seen in Figure 8 the earlier trends were further supported. The animals treated with ethanol/DNBS showed significantly higher signs of inflammation than control animals exposed to either ethanol or DNBS alone. Because of the lack of significance in TNF-α levels, this assay was not performed again at the 48 hour time point.

Figure 2. Results of testing mucosal integrity after treatment with ethanol. Evans Blue dye levels in rats with or without ethanol treatment were compared. Those treated with ethanol were significantly higher than the two other groups (significant $P < 0.05$).

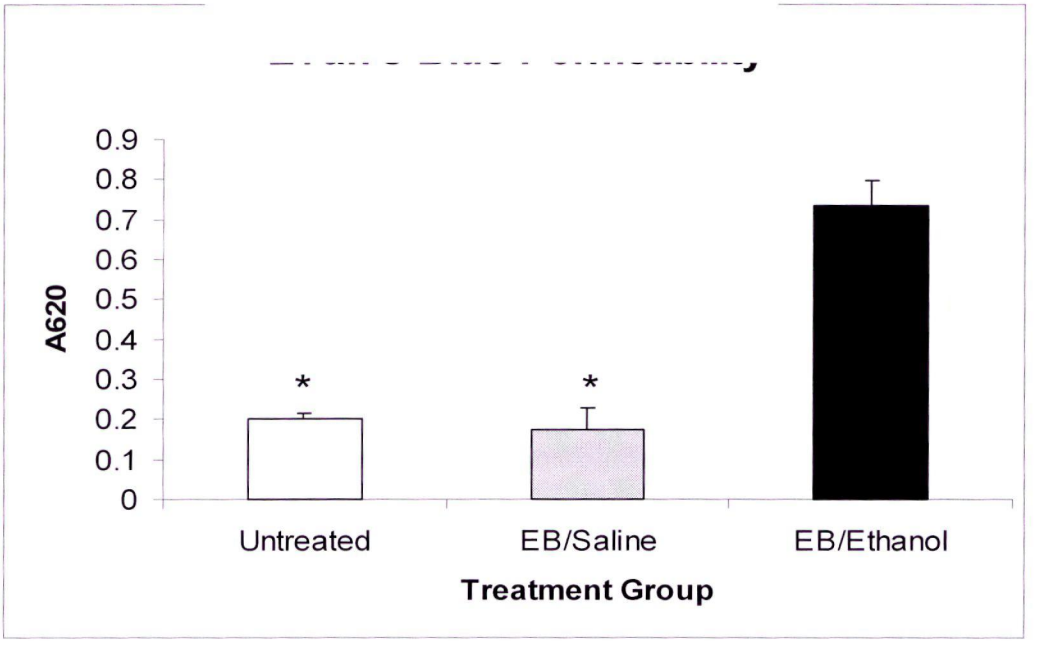

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
A620
*
*
Untreated
EB/Saline
EB/Ethanol
Treatment Group

Figure 3. Gross morphology scores for a time-course study of ethanol treated (EC), DNBS treated (DC), and ethanol/DNBS treated (T) rats. Untreated (UC) and bacterial prostatitis (BC) controls are included. Values from test animals (T) were compared to EC and DC results from the corresponding time groups (* = EC vs. T significant, ** = DC vs. T significant, $P < 0.05$).

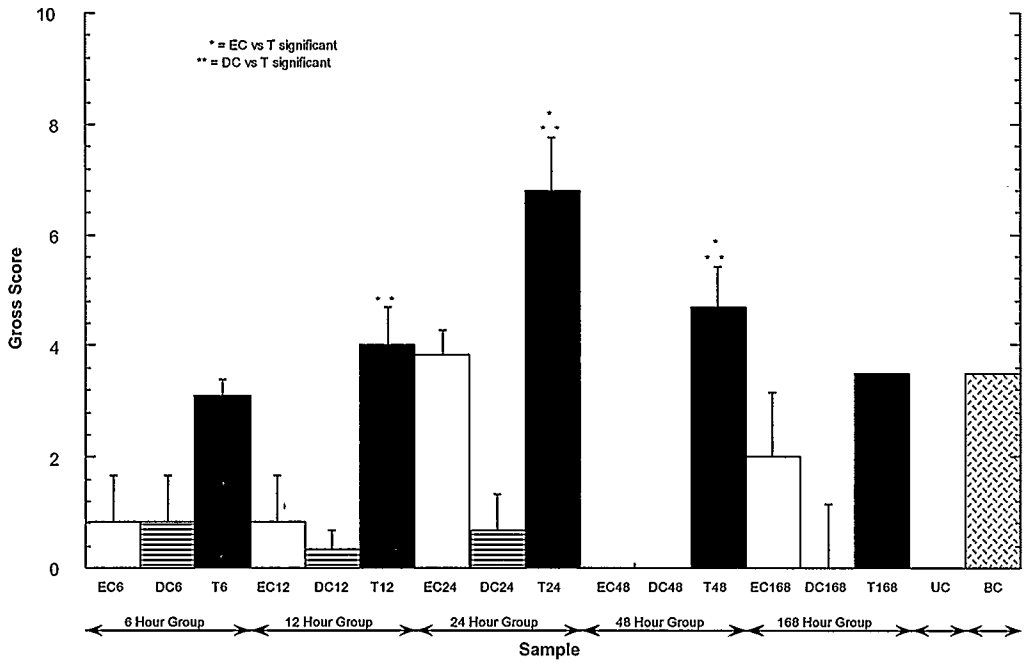

* = EC vs T significant
** = DC vs T significant
Gross Score
10
8
6
4
2
0
EC6
DC6
T6
EC12
DC12
T12
EC24
DC24
T24
EC48
DC48
T48
EC168
DC168
T168
UC
BC
6 Hour Group
12 Hour Group
24 Hour Group
48 Hour Group
168 Hour Group
Sample

Figure 4. Histology scores for a time-course study of ethanol treated (EC), DNBS treated (DC), and ethanol/DNBS treated (T) rats. Untreated (UC) and bacterial prostatitis (BC) controls are included. Values from test animals (T) were compared to EC and DC results from the corresponding time groups (* = EC vs. T significant, ** = DC vs. T significant, $P < 0.05$).

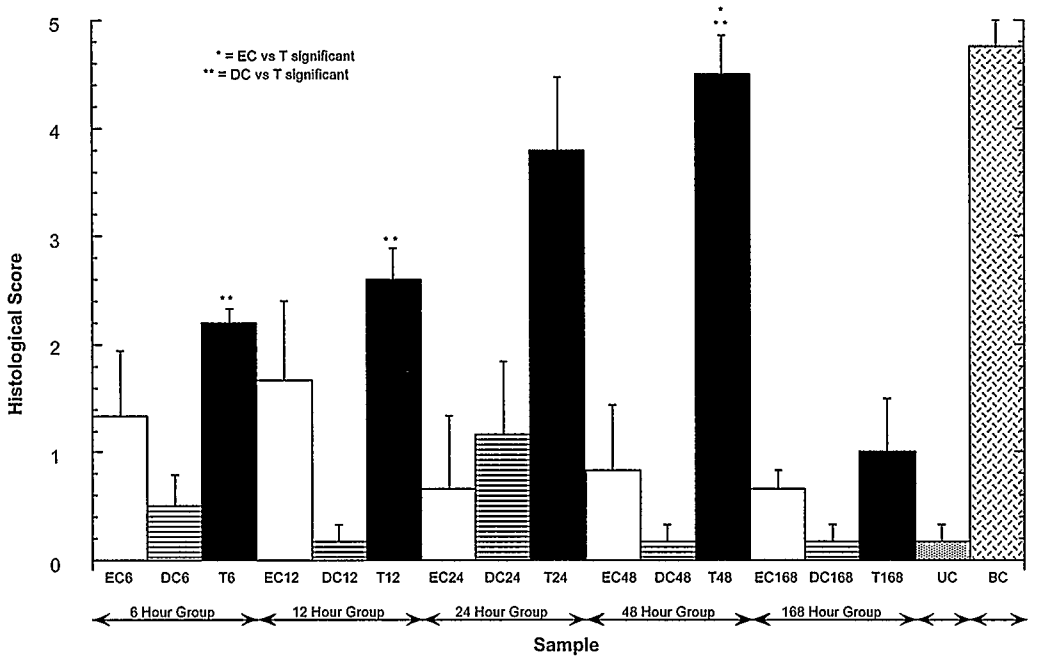

* = EC vs T significant
** = DC vs T significant
Histological Score
0
1
2
3
4
5
EC6
DC6
T6
EC12
DC12
T12
EC24
DC24
T24
EC48
DC48
T48
EC168
DC168
T168
UC
BC
6 Hour Group
12 Hour Group
24 Hour Group
48 Hour Group
168 Hour Group
Sample

Figure 5. Interleukin-1β concentrations for a time-course study of ethanol treated (EC), DNBS treated (DC), and ethanol/DNBS treated (T) rats. Untreated (UC) controls are included. Values from test animals (T) were compared to EC and DC results from the corresponding time groups (* = EC vs. T significant, ** = DC vs. T significant, $P < 0.05$).

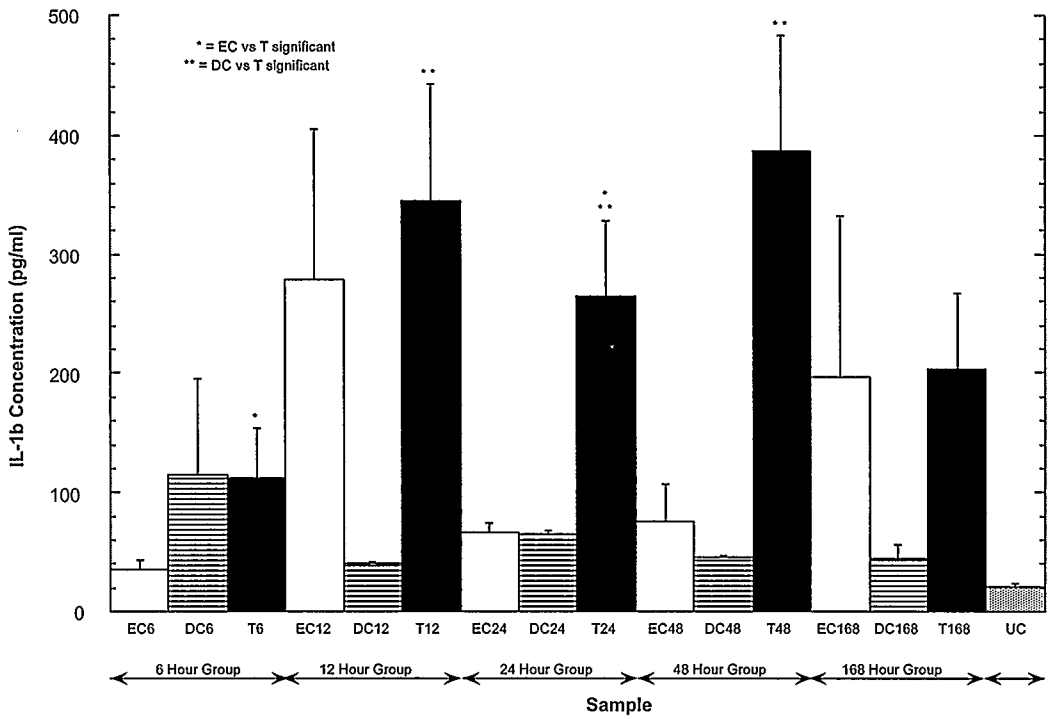

* = EC vs T significant
** = DC vs T significant
IL-1b Concentration (pg/ml)
500
400
300
200
100
0
EC6
DC6
T6
EC12
DC12
T12
EC24
DC24
T24
EC48
DC48
T48
EC168
DC168
T168
UC
6 Hour Group
12 Hour Group
24 Hour Group
48 Hour Group
168 Hour Group
Sample

Figure 6. Tumor necrosis factor-α concentrations for a time-course study of ethanol treated (EC), DNBS treated (DC), and ethanol/DNBS treated (T) rats. Untreated (UC) and bacterial prostatitis (BC) controls are included. Values from test animals (T) were compared to EC and DC results from the corresponding time groups.

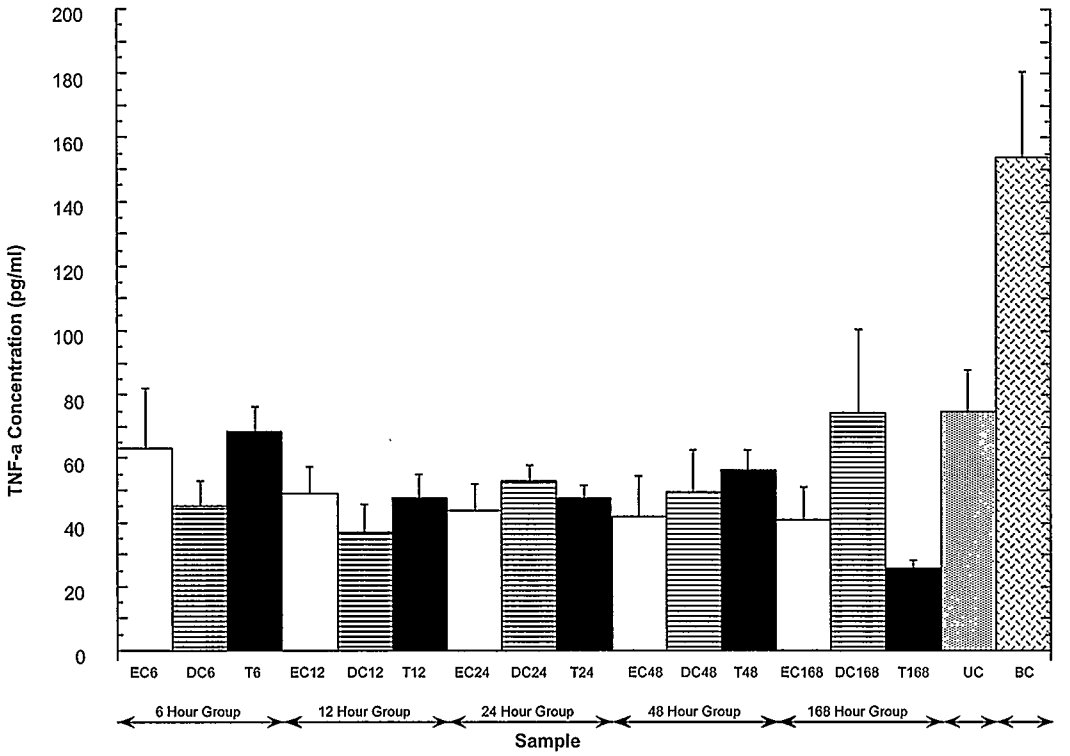

TNF-a Concentration (pg/ml)
200
180
160
140
120
100
80
60
40
20
0
EC6
DC6
T6
EC12
DC12
T12
EC24
DC24
T24
EC48
DC48
T48
EC168
DC168
T168
UC
BC
6 Hour Group
12 Hour Group
24 Hour Group
48 Hour Group
168 Hour Group
Sample

Figure 7. Cytokine levels at early time-points after catheterization with DNBS/ethanol (1 hr, 2 hr, 3 hr, and 4 hr).

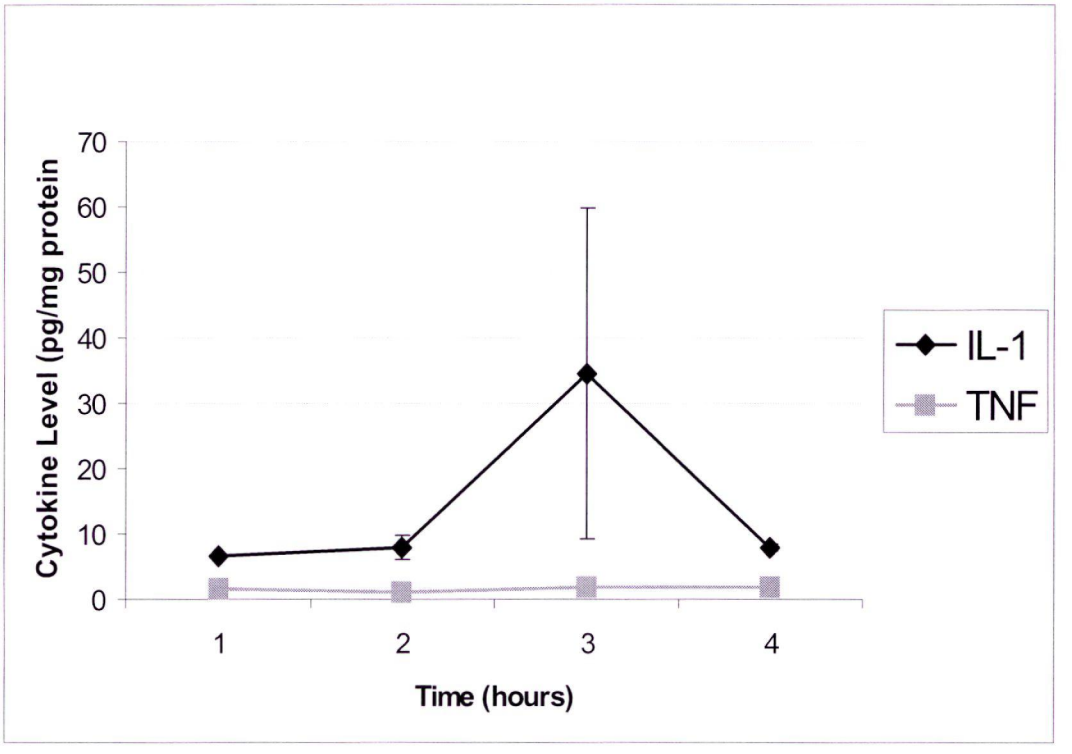
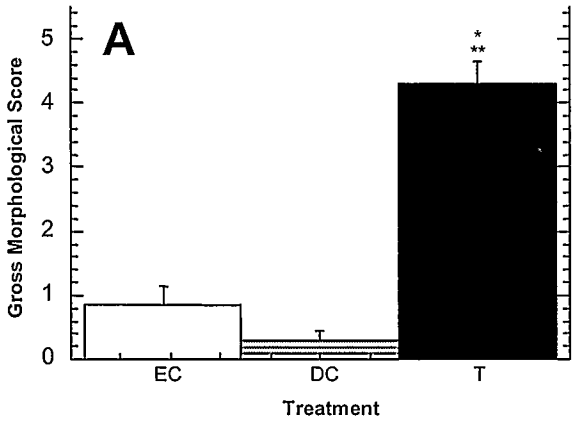
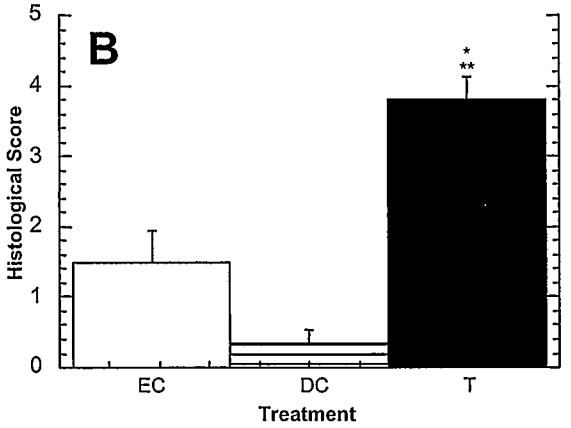


Figure 8. Gross morphology (A), histology (B), and interleukin-1β (C) scores for ethanol treated (EC), DNBS treated (DC), and ethanol/DNBS treated (T)

rats, sacrificed after 48 hours. Values from test animals (T) were compared to EC and DC results from the corresponding time groups (* = EC vs. T significant, ** = DC vs. T significant, $P < 0.05$).


A
Gross Morphological Score
0
1
2
3
4
5
EC
DC
T
Treatment
*
**


B
Histological Score
0
1
2
3
4
5
EC
DC
T
Treatment
*
**

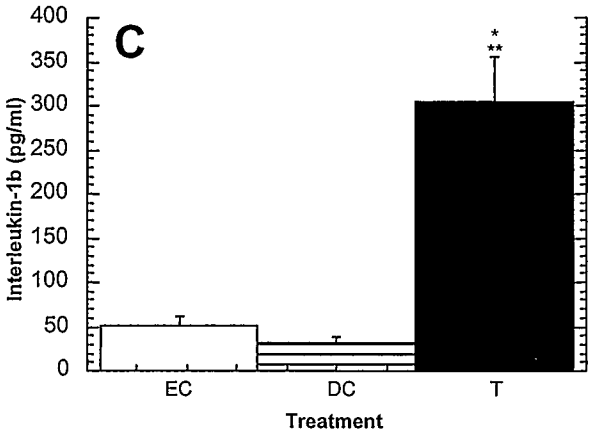
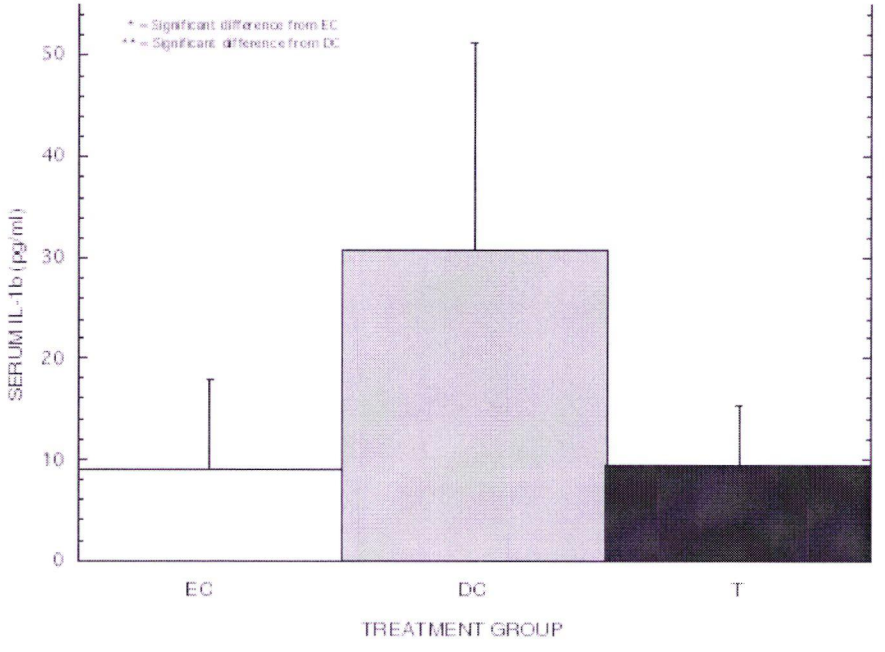

C
Interleukin-1b (pg/ml)
0
50
100
150
200
250
300
350
400
EC
DC
T
Treatment
*
**

Figure 9. Serum IL-1β in 48 hour test and control groups.

The levels of IL-1β were not statistically different in all 3 groups.


* = Significant difference from EC
** = Significant difference from DC
SERUM IL-1b (pg/ml)
0
10
20
30
40
50
EC
DC
T
TREATMENT GROUP

F. Serum IL-1β Levels

There was no significant difference in serum IL-1β levels in test and control rats (Fig. 9).

G. Bacterial Prostatitis CNF Model 1

1. Colonization

CP9 and CP9*cnf*$_1$ infected the prostate equally well. Prostates infected with CP9 contained an average of 1.3 x 10^6 CFU/mg tissue while those infected with CP9*cnf*$_1$ contained 7.4 x 10^5 CFU/mg tissue. This slight difference in bacterial numbers was not statistically significant. The experiment was repeated three times with different doses of CP9 or CP9*cnf*$_1$ with comparable results (data not shown).

Cystitis patient isolate C85 (O2:H, hemolysin-positive, CNF1-positive) and the isogenic mutant, C85*cnf*$_1$ (79), also reached equivalent levels of colonization in the prostate (7.1 × 10^4 and 4.9 × 10^4 CFU/mg of tissue,

respectively), although total bacterial numbers were less than those achieved with CP9 and its mutant (data not shown).

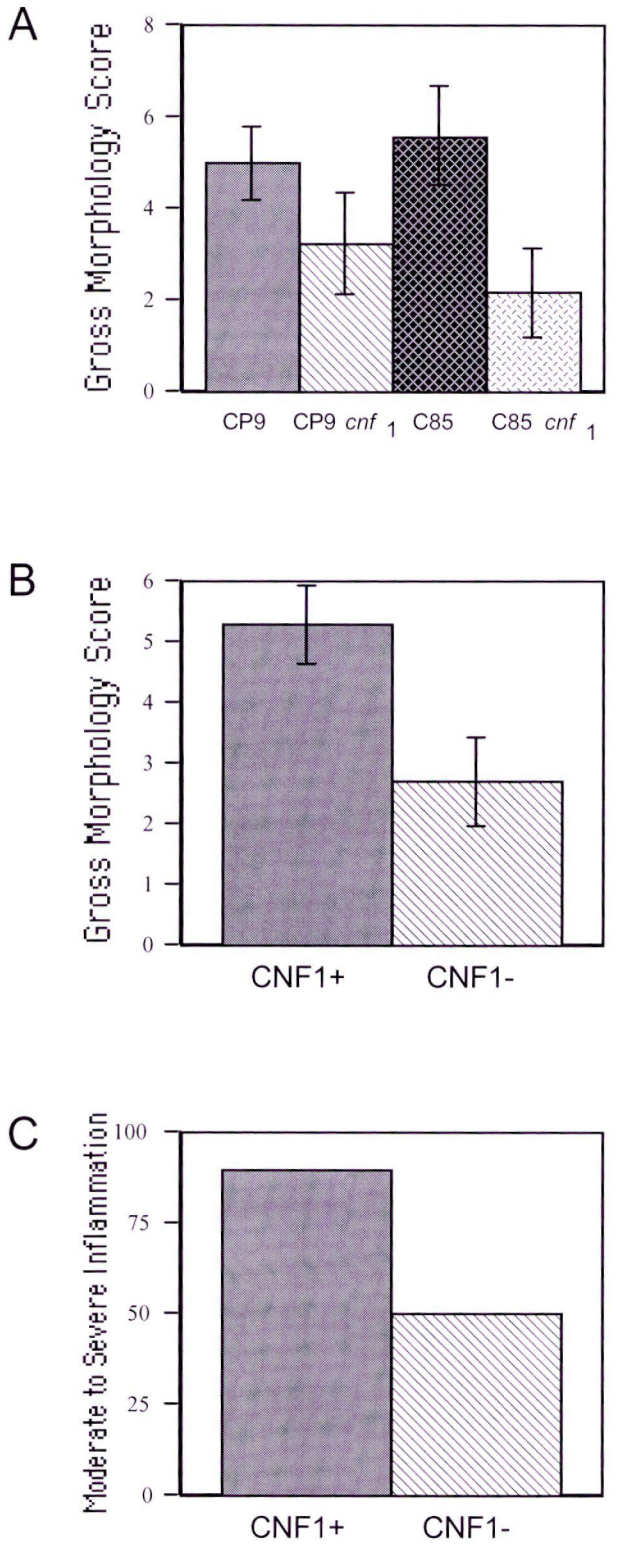
Next, we tried a mixed-infection experiment with a Lac-negative, CNF1-positive derivative of CP9 (CP9*lacZ*) and CP9*cnf*$_1$ (Lac-positive, CNF1 negative). Here, five rats were inoculated with a mixture that contained 2×10^7 CFU of each strain and were euthanatized 2 days later. Prostates homogenates were serially diluted and plated for colony counts. No difference in colony counts between the strains 2 days after inoculation was seen (9.3×10^5 CFU/mg of tissue for CP9*lacZ*-infected rats versus 6.7×10^5 CFU/mg of tissue for CP9*cnf*$_1$-infected rats) (Fig. 10). Thus, CNF1 does not appear to play a role in colonization of the prostate by a UPEC strain.

2. Gross Morphology

Prior to homogenization of the prostates for colony counts, the gross morphologies of all infected prostates ($n = 6$) were scored. Infected prostates were observed for overt signs of

Figure 10. CNF1-positive strains (CP9 and C85) cause more gross morphological damage to the prostate than CNF1-negative strains ($CP9_{cnf1}$ and $C85_{cnf1}$). Gross morphological changes were given a score of 0 to 3 for edema, congestion and hyperemia (0 was none, 3 was the maximum score). Data are from 6 rats for each strain. Scores were combined to give the total score which is depicted in the graph. Panel A. Average gross morphology scores for each individual strain. Panel B. Average gross morphology scores for combined CNF1-positive and combined CNF1-negative strains. Panel C. The percentage of animals displaying moderate (score of 2-4) to severe inflammation (score of 5-9) is shown on the graph.

inflammation, including edema, congestion, and hyperemia, and given a score of 0 to 3 for each of these conditions (0 = none; 3 = the maximum score). The three scores were then totalled for a final score of 0 to 9. Examination of the gross morphologies of the infected prostates as well as the gross pathology score revealed differences between the CNF1-positive and CNF1- negative strains (Fig. 10). CNF1 production by strains CP9 and C85 promoted an increase in the severity of both edema and hyperemia in the prostates (data not shown). Prostates of animals infected with CP9 exhibited a trend toward severe tissue damage,



while those infected with CP9*cnf*$_1$ had more moderate damage (Fig. 10). Similarly, those animals infected with C85 had statistically more severe damage to the prostate than did those infected with C85*cnf*$_1$ (one-way analysis of variance, $P = 0.015$) (Fig. 10). When the data from the two CNF1-positive strains were combined, the wild-type strains caused significantly more damage than did the isogenic mutants ($P < 0.05$) (Fig. 10). Thus, 90% of animals infected with CNF1-positive strains had moderate to high inflammation, evident upon examination of the gross morphology of the prostate (Fig. 10). Animals infected with CNF1-negative bacteria were less likely to exhibit moderate to high inflammation (50% of all animals) (Fig. 10).

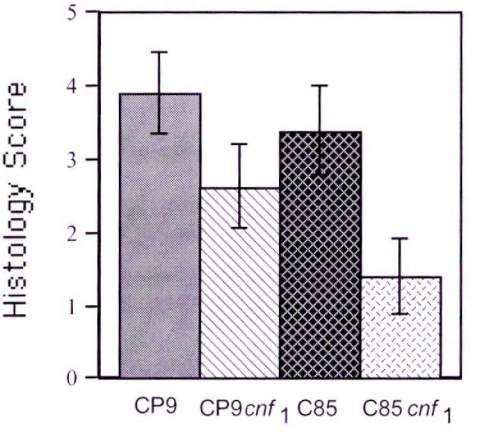
3. Histology

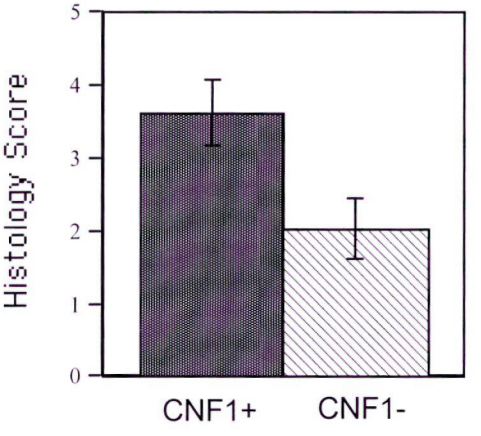
We then examined the influence of CNF1 production by UPEC on the histology of prostate tissues. Prostates infected with wild-type bacteria consistently showed histological evidence of more extensive and severe inflammation than did those infected with the CNF1-negative mutants (Fig. 11). Acini in prostates infected with CP9 were filled with neutrophils, and the stromal tissue was edematous. Conversely, the acini of prostates from CP9*cnf*$_1$-infected animals contained far fewer neutrophils and the stromal tissue was markedly less edematous.

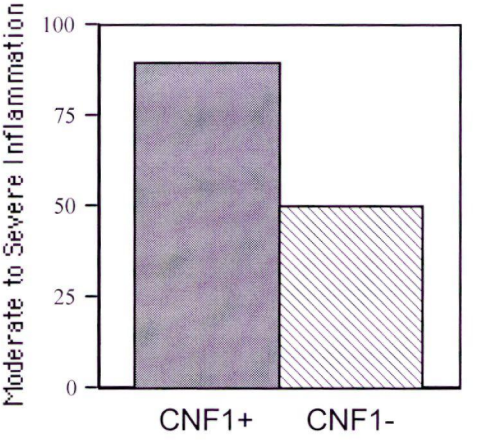
To more quantitatively measure the readily visible histological differences between prostates infected with CP9 and those infected with CP9*cnf*$_1$, stained sections were scored for histological signs of inflammation: edema, hemorrhage, and leukocyte infiltration. Total scores of less than 2 were classified as mild inflammation, scores of 2 to 4 were considered moderate inflammation, and scores of 5 or greater were categorized as severe inflammation. Tissue sections from prostates of animals infected with CP9 strains exhibited a trend toward more extensive damage, as measured by examination of the levels of edema, hemorrhage, and leukocyte infiltration, than did prostates infected with the isogenic mutant (Fig. 11).

Figure 11. CNF1-positive strains (CP9 and C85) cause more histological damage to the prostate than CNF1-negative strains (CP9$_{cnf1}$ and C85$_{cnf1}$). Slides were examined under light microscopy and each prostate was given a score of 0 to 3 for edema, hemorrhage and leukocyte infiltration (0 was none, 3 was the maximum score). Data are from six rats for each strain. Scores were combined to give the total score which is depicted in the graph. Panel A. Average histology scores for each individual strain. Panel B. Average histology

scores for combined CNF1-positive and combined CNF1-negative strains. Panel C. The percentage of animals displaying moderate (score of 2-4) to severe inflammation (score of 5-9) is shown on the graph.







While the histological damage caused by strain C85 was not as extensive as that caused by CP9, damage caused by strain C85 and damage caused by strain C85*cnf*$_1$ were statistically different ($P < 0.05$) (Fig. 11). When histology data for all prostates infected with CNF1-positive bacteria were combined, these prostates were more damaged than were prostates infected with the isogenic mutants ($P < 0.05$) (Fig. 11). All animals infected with strain CP9 had moderate to high inflammation in comparison to 66% of those infected with CP9*cnf*$_1$ (Fig. 11). Strain C85 caused severe damage slightly less often than did strain CP9, with 80% of wild-type- and only 33% of mutant-infected prostates manifesting moderate to high inflammation (Fig.11). In sum, prostates infected with either wild-type strain, CP9 or C85, had more extensive gross tissue damage, as well as more severe histological damage, than did prostates infected with either mutant strain.

The impact of CP9 and C85 differed. C85 caused significantly more damage to the prostate than did its mutant, while the differences between CP9 and its mutant were not significant.

J. Bacterial Prostatitis CNF Model 2

1. Colonization

As seen in Figure 12, due to the large variation within groups there was no statistical difference in the colonization abilities of CP9 and CP9*cnf*$_1$. While not a significant difference, one can see that CP9 did show a slightly greater ability to colonize the prostates.

2. Gross morphology

Observations of gross morphological changes to the infected prostates revealed that the presence of CNF1 leads to increased inflammatory damage. Overall gross morphology scores were significantly higher in CP9-infected animals (one-way analysis of variance, P = 0.015) (Fig. 13). The percentage of animals showing severe inflammation (gross scores of 5 to 9) was markedly higher in the CP9 treatment group (Fisher's Exact test, P = 0.008) (Fig. 13).

3. Histology

To further examine the extent of the inflammatory damage, routine histology was performed. Scoring the infected prostates revealed a general trend of higher inflammatory damage from CP9

Figure 12. The lack of CNF1 did not significantly affect the colonization ability by CP9*cnf*$_1$ (WEX135). Data is expressed as CFU per mg of prostatic tissue.

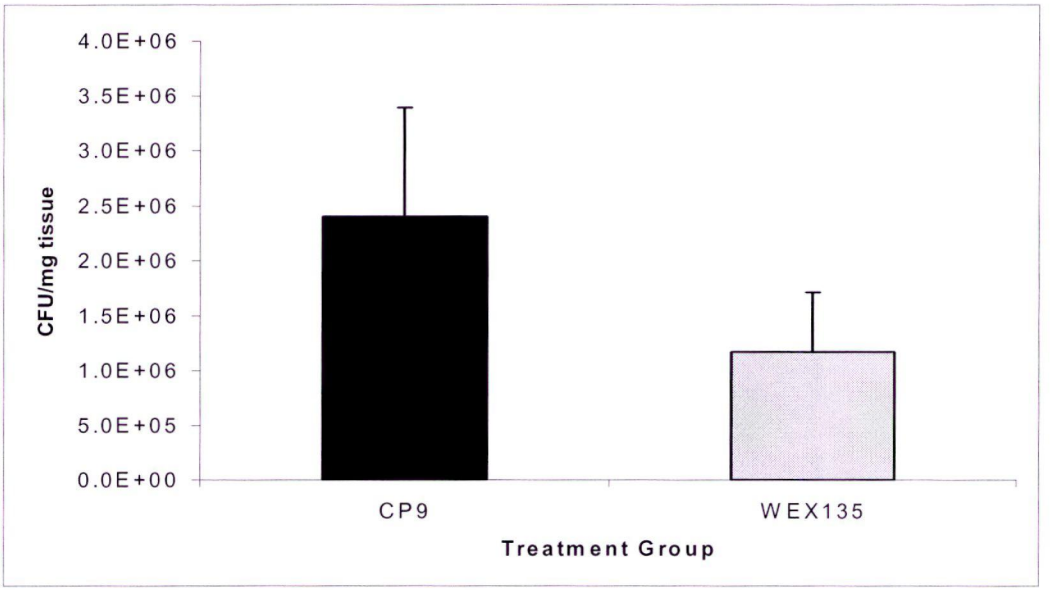
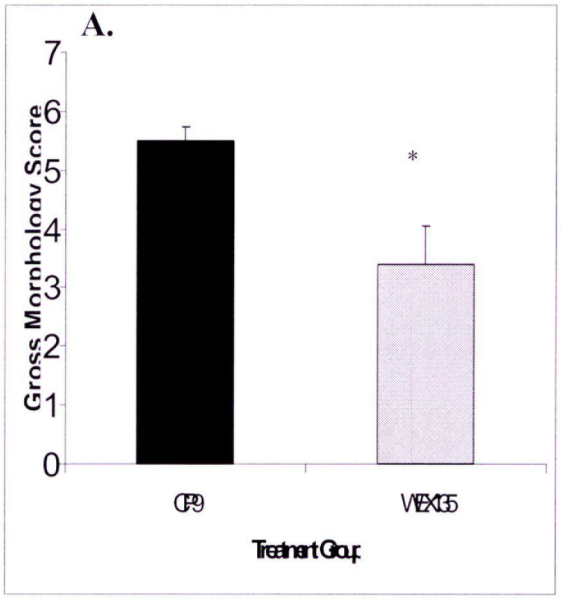

4.0E+06
3.5E+06
3.0E+06
2.5E+06
2.0E+06
1.5E+06
1.0E+06
5.0E+05
0.0E+00
CFU/mg tissue
CP9
WEX135
Treatment Group

Figure 13. Infection with CNF1-positive *E. coli* CP9 leads to greater gross morphological prostatic damage than does its isogenic CNF1-negative strain CP9*cnf*$_1$. Prostate gross morphology was assayed with edema, hyperemia and congestion each receiving a score of 0 to 3. These scores were combined to give a total gross morphology score out of 9. (A) Average gross morphology scores for each strain. Treatment groups showed a statistical difference by one-way analysis of variance, P = 0.015. (B) Percentages of animals that displayed severe inflammation (score of 5 to 9). Treatment groups showed a statistical difference by Fisher's Exact test, P = 0.008.


A.
Gross Morphology Score
7
6
5
4
3
2
1
0
*
CF9
VEX35
Treatment Group

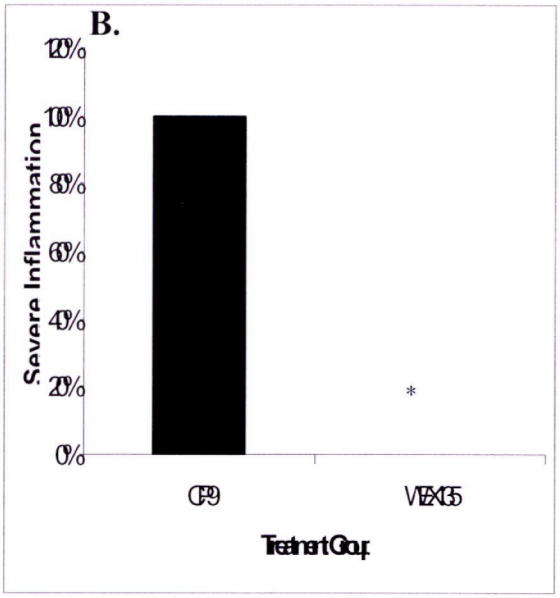
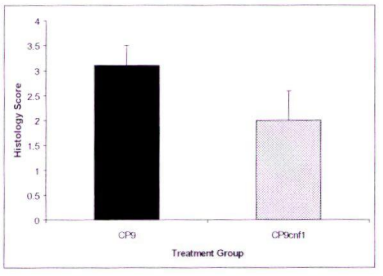
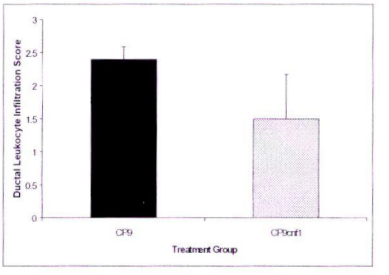
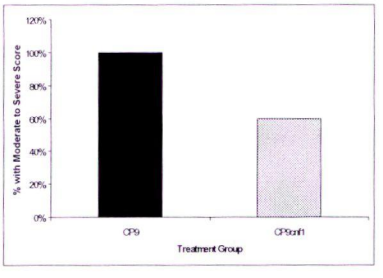
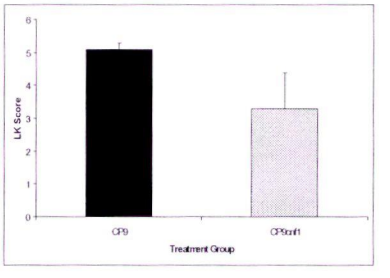

B.
Severe Inflammation
120%
100%
80%
60%
40%
20%
0%
*
CF9
VEX35
Treatment Group

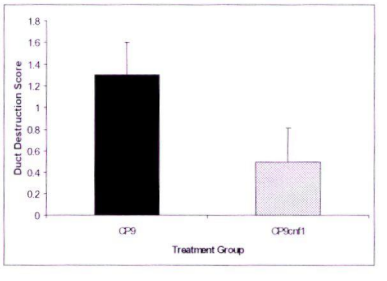
Figure 14. The presence of CNF1 enhanced histological damage in the prostate. Edema, hemorrhage and leukocyte infiltration were each assessed a score of 0 to 3 and the three scores were totaled to obtain a total histological damage score out of 9. (A) Average histological scores for each strain. To more specifically assay the leukocyte involvement, interstitial leukocytes, ductal leukocyte infiltration, and duct destruction were each assigned a score of 0 to 3 and these three scores were totaled to obtain a final leukocyte score out of 9. (B) Average leukocyte score for each strain. (C) Percentages of animals that displayed moderate to severe inflammation (score of 2 to 9). (D) Average ductal leukocyte infiltration. (E) Average duct destruction.


Histology Score
4
3.5
3
2.5
2
1.5
1
0.5
0
CP9
CP9cnf1
Treatment Group


Ductal Leukocyte Infiltration Score
3
2.5
2
1.5
1
0.5
0
CP9
CP9cnf1
Treatment Group


% with Moderate to Severe Score
120%
100%
80%
60%
40%
20%
0%
CP9
CP9cnf1
Treatment Group


LK Score
6
5
4
3
2
1
0
CP9
CP9cnf1
Treatment Group


Duct Destruction Score
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
CP9
CP9cnf1
Treatment Group

than CP9*cnf*$_1$ but the differences were not statistically significant (Fig. 14). The leukocyte scores demonstrate a trend of more severe damage resulting from the presence of CNF1 (Fig. 14). Closer examination reveals the bulk of the histological difference was due to ductal infiltrate by leukocytes and subsequent duct destruction (Fig. 14).

4. Myeloperoxidase

Because of the leukocytic infiltrate into the prostate tissue, MPO levels were measured in tissue homogenates to compare relative neutrophil levels. As seen in Figure 15, CP9 caused a significantly higher neutrophil accumulation, seen as increased MPO, in the prostate than did CP9*cnf*$_1$ (one-way analysis of variance, $P = 0.039$).

5. Inflammatory Cytokines

A panel of pro-inflammatory mediators was chosen for assay to gain more insight into the influence of CNF1 on prostatic inflammation. As seen in Figure 16, there is a common trend detailing an increased level of IL-1β, GRO/CINC-1, LTB_4 in tissues exposed to CP9 compared to those challenged with CP9*cnf*$_1$. While

Figure 15. Prostatic tissue of animals infected with CP9 showed higher levels of MPO than those infected with CP9*cnf*$_1$. Treatment groups showed a statistical difference by one-way analysis of variance, P = 0.039.

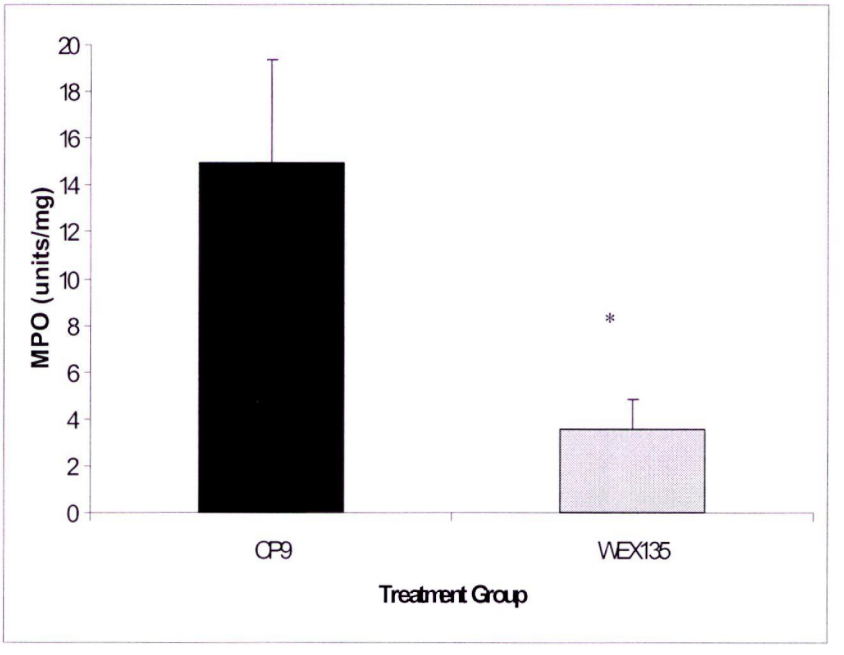
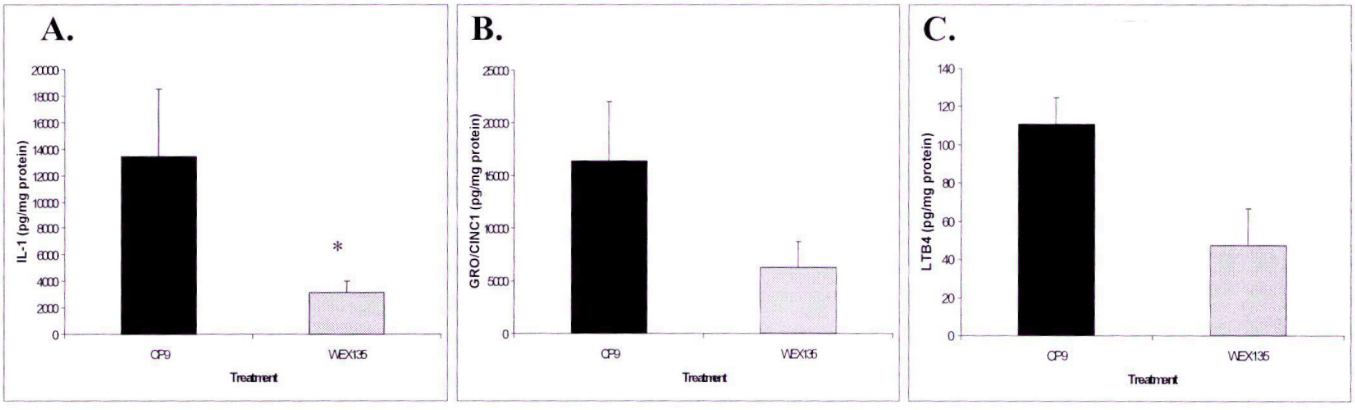

20
18
16
14
12
10
8
6
4
2
0
MPO (units/mg)
*
CP9
WEX135
Treatment Group

Figure 16. Pro-inflammatory mediators were higher in prostates exposed to CP9 versus those exposed to CP9*cnf*$_1$. (A) IL-1β levels in prostatic tissue exposed to either strain. (B) GRO/CINC-1 levels in prostatic tissue. (C) LTB_4 levels in prostatic tissue.


A.
IL-1 (pg/mg protein)
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
*
CP9
WEX135
Treatment
B.
GRO/CINC1 (pg/mg protein)
25000
20000
15000
10000
5000
0
CP9
WEX135
Treatment
C.
LTB4 (pg/mg protein)
140
120
100
80
60
40
20
0
CP9
WEX135
Treatment

these differences were not statistically significant, the levels of these molecules were all higher in CP9-infected animals.

G. Abacterial Prostatitis IL-1Ra Model

From the results of our current experiments, we are led to believe that IL-1β may play a significant role in the development of inflammation in our model of abacterial prostatitis. We are now interested in determining if inhibiting the action of IL-1β can in turn lessen the extent of the inflammation in our model.

Figure 17 summarizes the data for the assays of inflammation. The control group catheterized with saline and injected with IL-1Ra consistently displayed the lowest inflammation scores, due to the lack of the barrier breaker and irritant.

1. Gross Morphology and Histology

To examine the inhibitory effect of IL-1Ra, we compared the results from rats catheterized with DNBS/ethanol and injected with either IL-1Ra or saline. As seen in Figure 17, the gross morphological scores were not markedly different between these groups, while the histological scores (Fig. 18) did show significant decreases in the IL-1Ra group.

2. Inflammatory Mediators

The levels of IL-1β (Fig. 19) were not statistically different, but were very nearly so (P= 0.055). LTB_4 (Fig. 20), GRO/CINC (Fig. 21) and myeloperoxidase (Fig. 22) also showed significant decreases in the IL-1Ra group.

Overall the receptor antagonist treatment produced a decrease in inflammation, not to the levels of healthy controls, but consistently lower than those shown in animals catheterized with DNBS/saline but not receiving the IL-1Ra.

Figure 17. Gross morphology results in the abacterial prostatitis IL-1Ra model. Note the difference between second and third groups.

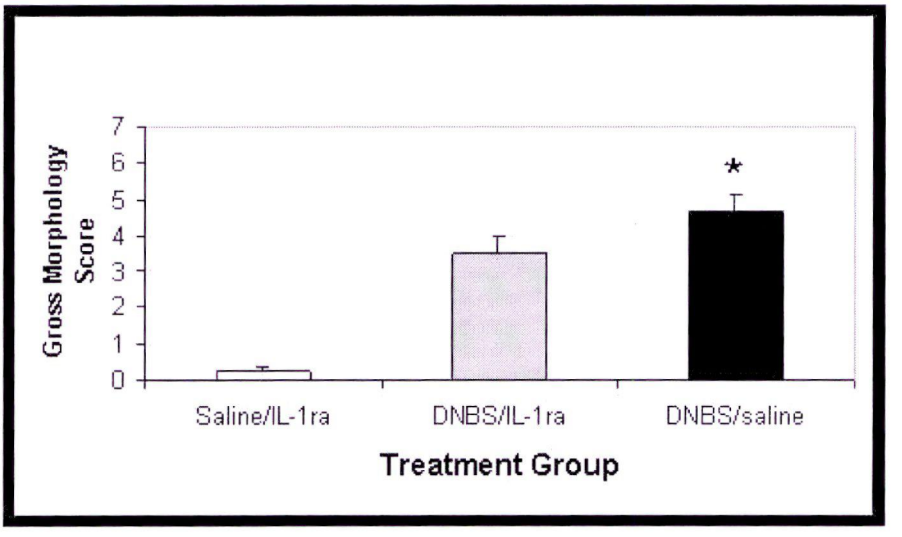

Gross Morphology Score
0
1
2
3
4
5
6
7
*
Saline/IL-1ra
DNBS/IL-1ra
DNBS/saline
Treatment Group

Figure 18. Histology results in the abacterial prostatitis IL-1Ra model. The IL-1Ra treatment resulted in significantly less damage (columns 2 and 3).

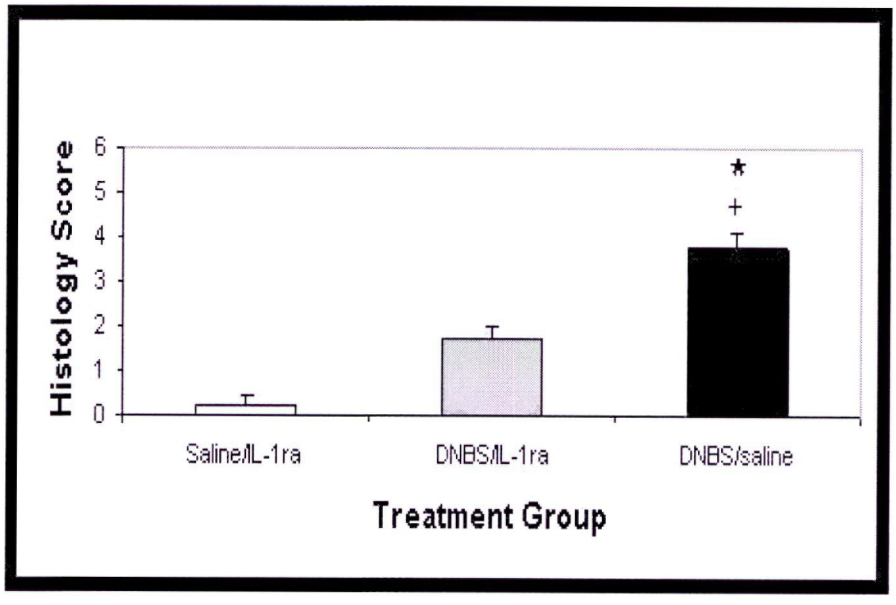

Histology Score
6
5
4
3
2
1
0
*
+
Saline/IL-1ra
DNBS/IL-1ra
DNBS/saline
Treatment Group

Figure 19. IL-1β levels seen in the abacterial prostatitis IL-1Ra model. Animals exposed to ethanol showed increased IL-1β over the saline control. The IL-Ra treated animals showed a lower IL-1β response than the group not receiving IL-1Ra.

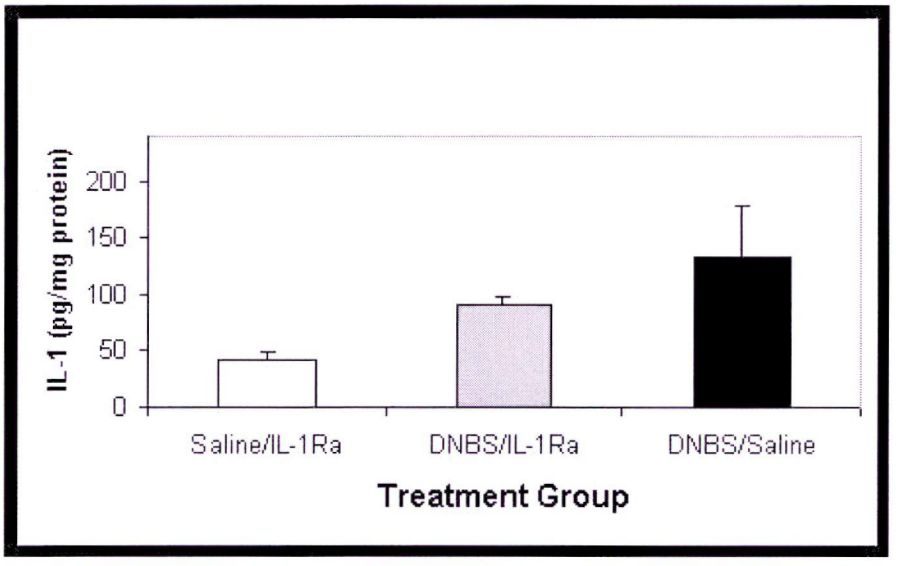

IL-1 (pg/mg protein)
200
150
100
50
0
Saline/IL-1Ra
DNBS/IL-1Ra
DNBS/Saline
Treatment Group

Figure 20. LTB_4 levels seen in the abacterial prostatitis IL-1Ra model.

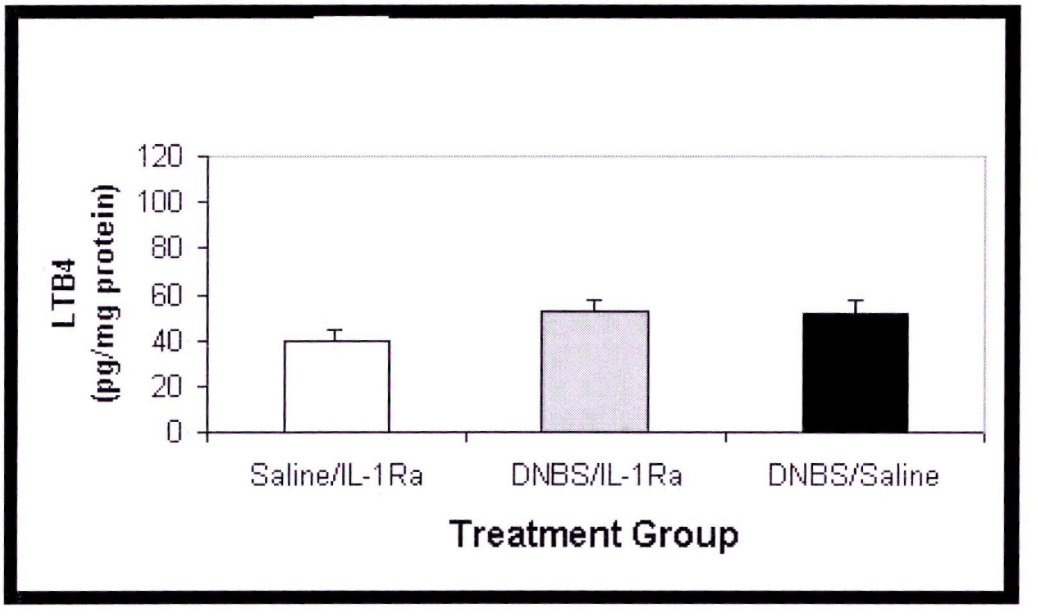

LTB4
(pg/mg protein)
120
100
80
60
40
20
0
Saline/IL-1Ra
DNBS/IL-1Ra
DNBS/Saline
Treatment Group

Figure 21. GRO/CINC levels seen in the abacterial prostatitis IL-1Ra model. The IL-1Ra treated group still had a GRO/CINC response higher than the control, but this response was much lower than in the group not receiving IL-1Ra.

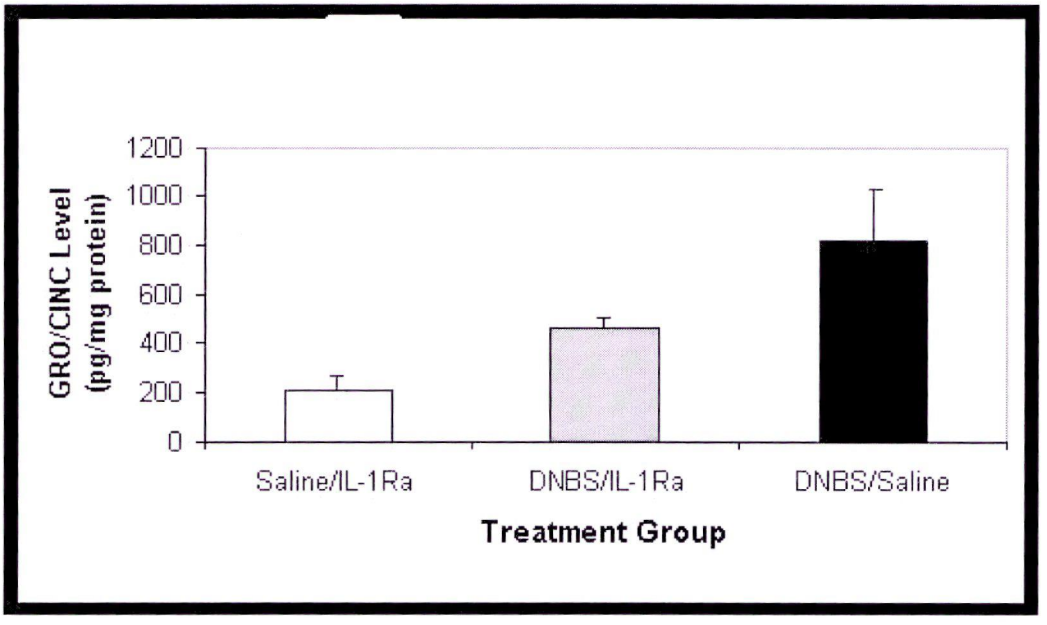
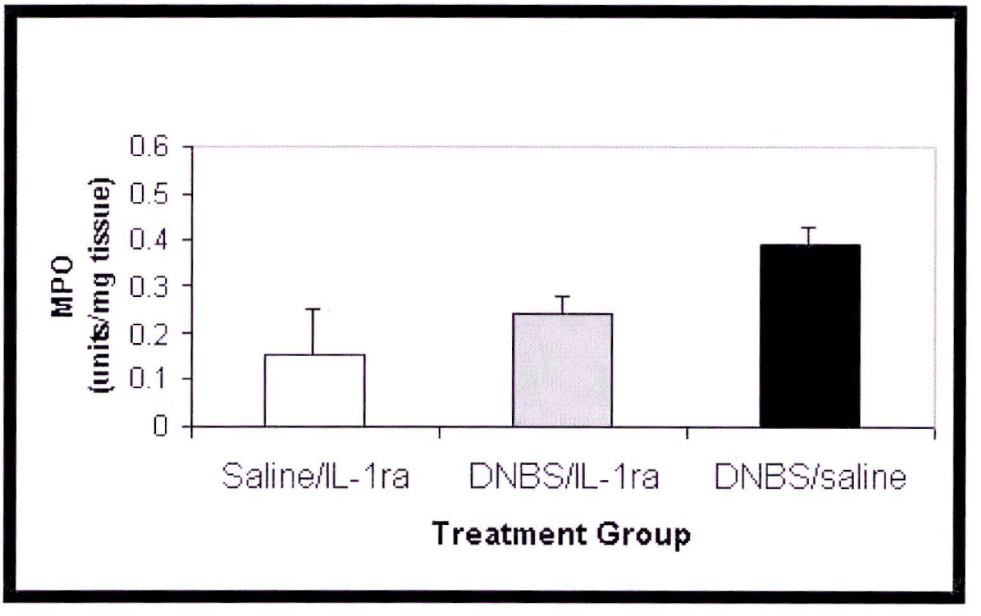

GRO/CINC Level
(pg/mg protein)
1200
1000
800
600
400
200
0
Saline/IL-1Ra
DNBS/IL-1Ra
DNBS/Saline
Treatment Group

Figure 22. MPO levels seen in the abacterial prostatitis IL-1Ra model. Much like the pattern seen in the other inflammatory responses, the MPO results indicate a decrease in MPO in animals given IL-1Ra.


MPO
(units/mg tissue)
0.6
0.5
0.4
0.3
0.2
0.1
0
Saline/IL-1ra
DNBS/IL-1ra
DNBS/saline
Treatment Group

H. Bacterial Prostatitis IL-1Ra Model

The results of this study were inconclusive (Figs. 23-26). Gross morphology scores, levels of IL-1β, and levels of myeloperoxidase were not significantly different between the two groups. The levels of variation within groups were very high, suggesting the model itself was inconsistent. The bacterial model results in levels of IL-1β much higher than those seen in the abacterial model so it seems fitting that more IL-1Ra should be used in the bacterial model in future studies.

Figure 23. Bacterial prostatitis model with IL-1Ra: Gross morphology scores for control and test rats in the moderate to severe range. No difference was appreciated between the two groups.

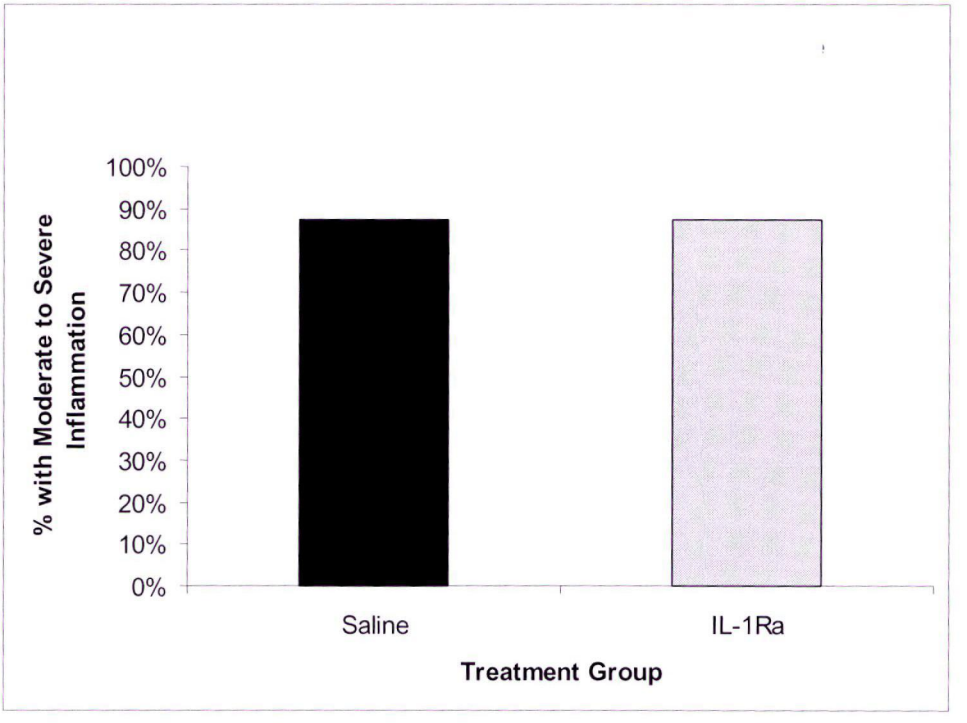

% with Moderate to Severe Inflammation
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Saline
IL-1Ra
Treatment Group

Figure 24. Bacterial prostatitis model with IL-1Ra: Gross morphology scores for control and test rats in the severe range. Here we see a slightly enhanced inflammatory result in those treated with IL-1Ra.

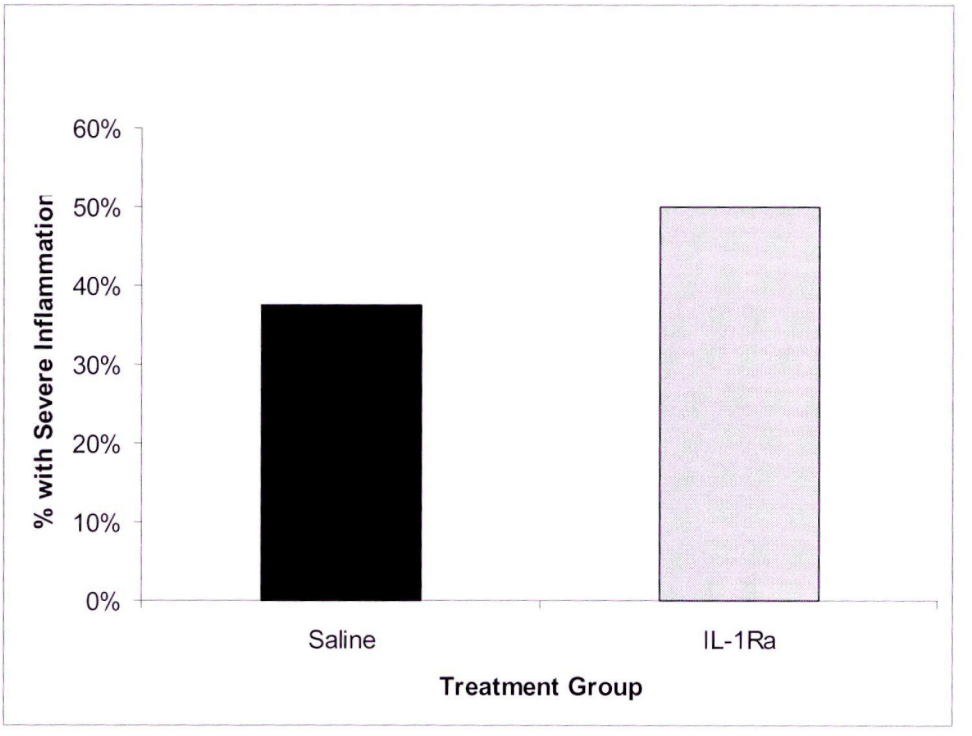

60%
50%
40%
30%
20%
10%
0%
% with Severe Inflammation
Saline
IL-1Ra
Treatment Group

Figure 25. Bacterial prostatitis model with IL-1Ra: IL-1β levels in prostate tissue of test and control rats. Levels of IL-1β in the IL-1Ra treated group showed a higher level of this inflammatory marker than that seen in the control group.

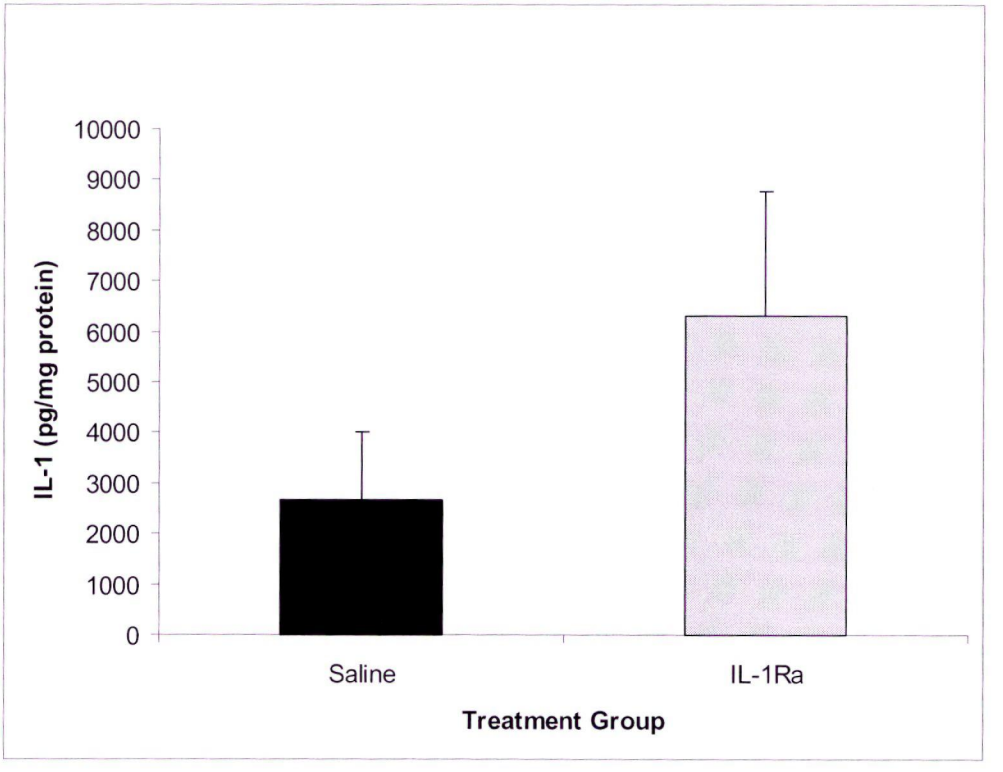
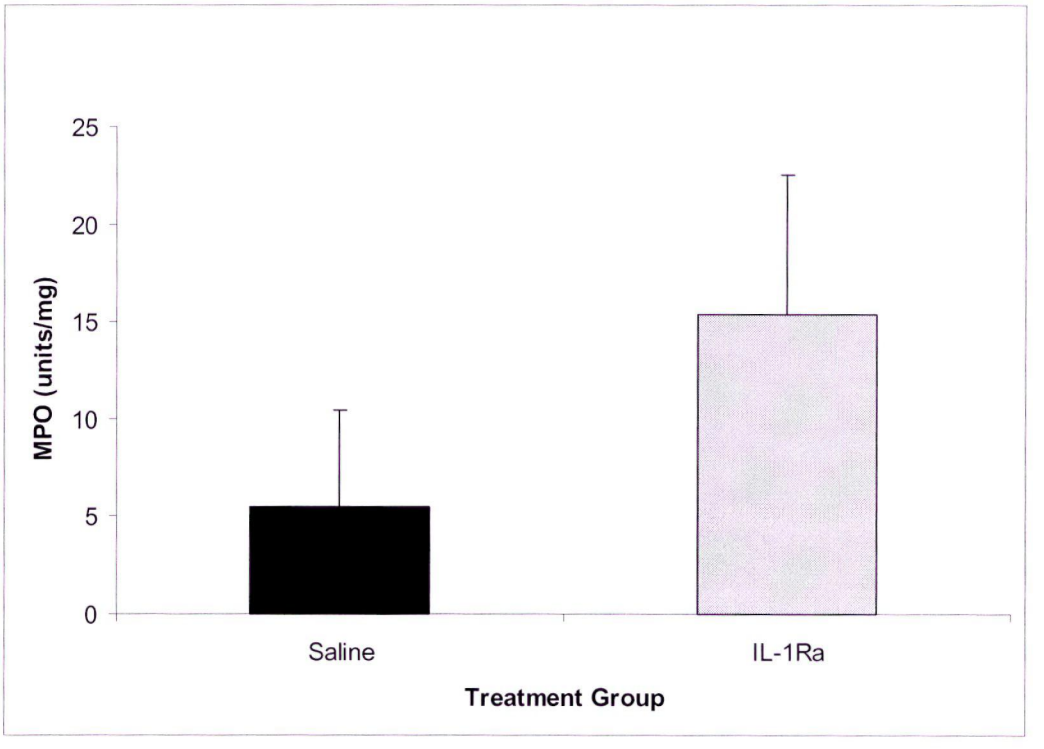

10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
IL-1 (pg/mg protein)
Saline
IL-1Ra
Treatment Group

Figure 26. Bacterial prostatitis model with IL-1Ra: MPO levels in prostate tissue of test and control rats. Again, the IL-1Ra treated test group showed higher levels of inflammatory markers than did the control group.


25
20
15
10
5
0
MPO (units/mg)
Saline
IL-1Ra
Treatment Group

IV. Conclusions and Future Directions

A. Lack of Abacterial Model

The unknown etiology of abacterial prostatitis has led to the development of a number of animal models to study individual theories. Models evaluating spontaneous, age- and genetic-dependent prostatitis (56, 61) and bacterial prostatitis (50, 66) were followed by models demonstrating the autoimmune characteristics of the inflammation (84, 88). The model described in this document examined the pathogenesis of inflammation from a mucosal surface perspective.

The prostate gland is composed of ducts that are lined by a mucus-secreting epithelium permeating a fibro-muscular stroma. This mucus layer protects the underlying tissue from exposure to potentially harmful agents in the urine that may reflux up into the prostate. A deficiency in the protection provided by this mucus is hypothesized to predispose the ducts of the prostate to epithelial damage, possibly leading to inflammation. The damage may be physical or chemical in nature, or may even have an autoimmune stimulus that perpetuates the inflammation. We believe that the state of the mucosal blanket in the prostatic ducts plays an integral role in the initiation of prostatitis, no matter what the agent that causes the actual inflammation.

B. Using Models to Focus on Specific Molecular Events

1. Abacterial Prostatitis

In previous studies of inflammatory bowel disease, rat models of Crohn's disease and colitis were established using ethanol to compromise the mucosal surface and TNBS (or DNBS) to act as an irritant inducing inflammation (55). Using cultured mucosal cells, it has further been shown that ethanol stimulates the rapid release and exhaustion of intercellular mucus-molecule pools (86). Our data is consistent with this theory, showing that disruption of mucosal integrity by a barrier breaker (ethanol) was required for the irritant to gain access to prostatic epithelium in order to incite an inflammatory response (86). This ultimately leaves the epithelium with no mucosal barrier, allowing irritants access to underlying cells. The epithelial cells may heal and commence normal secretory function, but by that time the DNBS will have incited an inflammatory response. Through morphological and histological observations, we have shown significant increases in signs of inflammation in animals subjected to ethanol and DNBS (Fig. 8).

The ELISA results support this data with a quantifiable and well established marker of inflammation in interleukin-1β. Significant increases of this cytokine were seen in the test groups (Fig. 5). Parallels between the three assays of inflammation (gross morphology, histology, and IL-1β) can be seen,

especially at the 12 hour, 24 hour, and 48 hour time groups. This suggests an establishment of acute inflammation at these time points, reliant on the combination of a decline in mucosal protection with the challenge by an irritant. This combination of ethanol and DNBS is important, evident in the low levels of inflammation seen in the two control groups (EC and DC) over the same periods. These results were expanded upon at the 48 hour time point, confirming the significant increases in acute inflammation due to the instillation of both ethanol and DNBS.

TNF-α is a proinflammatory cytokine often linked with IL-1β in a synergistic effect. We demonstrated the independence of IL-1β and TNF-α in this model of inflammation. While IL-1β increased in the test animals (Fig. 5), the TNF-α levels did not significantly differ between control and test animals (Fig. 6). This suggests an uncoupling of the common IL-1β/TNF-α coordination in this model of abacterial prostatitis, with IL-1β remaining the major proinflammatory cytokine. In bacterial prostatitis however, TNF-α was seen to increase in conjunction with IL-1β.

The link between inflammation and loss of mucosal surface ultrastructure is not unique to the prostate. Other model systems, such as those for colitis and interstitial cystitis, have been used to show this (59, 78). One important model of colitis involves the strategy utilized in this study (55). It has been reliably proven that a barrier breaker in conjunction with a chemical irritant can incite inflammation in the colon. Enteric bacteria at the

site of ethanol/DNBS addition presumably have access to underlying tissues. While many researchers believed that the prostate is a sterile organ, this concept has been recently challenged (65) and it appears very likely that there is an autochthonous flora of the prostate that may become pathogenic under certain conditions, such as a break in mucosal integrity. Alternatively, potentially harmful substances in the urine refluxing into the prostate ducts (6) may take advantage of a reduced mucosal barrier within the prostatic ductal system, resulting in a nonspecific chemical or immunological inflammation. Another parallel model of idiopathic inflammation has been developed for interstitial cystitis of the bladder. Irrigation with protamine sulfate (73) or a combination of protamine sulfate and urea or potassium (68) has been shown to disrupt the mucosal lining of the bladder, leading to inflammation similar to that of interstitial cystitis. Our model varies from the protamine sulfate interstitial cystitis models, in that we do not observe the characteristic denudation of the epithelium brought on by protamine sulfate instillation in the bladder (68, 73).

Using a model of ethanol/DNBS-mediated mucosal injury, we have been able to establish acute abacterial prostatitis in rats. Disruption of mucosa with ethanol alone was not enough to consistently create a significant inflammation, nor was addition of DNBS without a barrier breaker. We have therefore confirmed an artificial disruption of the mucosal barrier can allow irritants present in the prostatic acini to incite an inflammatory reaction. Variations in

mucosal integrity may play a pivotal role in the pathogenesis and perpetuation of inflammation in the prostate gland.

Our studies have shown an elevation in interleukin-1β in this model of abacterial prostatitis. We demonstrated a role for IL-1β in this model using IL-1Ra as an inhibitor, revealing a decrease in the signs of inflammation. The reduction in inflammation was not to the level seen in animals not given DNBS/ethanol, but it was intermediate between those and inflamed animals not receiving IL-1Ra. The low levels of inflammation in saline-catheterized, IL-1Ra-injected animals suggests the administration of the antagonist has no inflammatory effects.

While the decrease in inflammation due to IL-1Ra suggests IL-1β plays a role in this model, the evidence supports the theory that some other inflammatory mediators must also be involved. Our work suggests TNF-α has no key role, so it remains to be found which mediators are additive to the IL-1β.

2. Bacterial Prostatitis

Even though the presence of CNF1 appeared to augment inflammation in the prostates of rats, the impact of CP9 and C85 differed. C85 caused significantly more damage to the prostate than did its mutant, while the differences between CP9 and its mutant were not significant. This is in contrast

with cystitis findings (84), where the differences between CP9 and its mutant were greater than those between the C85 strain pair. CP9 and C85 were isolated from different sources within the body and have different repertoires of virulence factors. These variations in sources of strain isolation and virulence factor constellations may account for the observed differences between damage mediated by CP9 and that mediated by C85 in the bladder or prostate.

In these experiments, we tested an acute model of bacterial prostatitis because we had observed that CNF1 plays an early role in establishing infection and promoting inflammation in the mouse bladder (79). We did not evaluate the influence of CNF1 in chronic prostatitis. Because bacterial 16S ribosomal DNA sequences similar to those of *E. coli*, but not viable bacteria, have been found in the prostates of men with chronic prostatitis as well as in men with prostate cancer (45), we intend to extend our investigation on the role of CNF1 in chronic prostatitis. Furthermore, it is conceivable that CNF1, through deamidation and activation of the Rho family of small GTPases (29, 49, 89), is involved in development of prostate cancer. Constitutive activation of the Rho GTPases by CNF1 in the prostate would result in aberrant cell signalling which could, in turn, lead to transformation of the cell. Indeed, activation of the Rho GTPases has been shown to weakly transform fibroblast cell lines, and a role for RhoA and Rac1 in Ras transformation has been established elsewhere (44, 76). Rho GTPase signalling in prostate cell lines has

not been examined, and so it is possible that CNF1-positive UPEC could contribute to the development of prostate cancer. Whether further studies support such a speculative hypothesis, the findings in this report are the first to our knowledge that show a definitive role for any UPEC virulence factor in acute prostatitis in an animal model.

The results of this study support the role of CNF1 in an animal model of bacterial prostatitis (78). Initial research has shown that uropathogenic *E. coli* capable of producing CNF1 caused enhanced inflammatory damage to the prostate tissue of male rats, as compared to animals exposed to a CNF1 isogenic mutant. Then we expanded upon that study, focusing more specifically on the inflammatory reaction. Gross morphology, histological presentation, cellular recruitment, and pro-inflammatory mediator levels were assayed and conformed to a general trend. Animals infected with CNF1-positive UPEC showed generally higher amounts of inflammatory damage, neutrophil infiltration, and pro-inflammatory mediator levels. The enhanced neutrophil recruitment could have lead to the increased inflammatory damage revealed in this study.

A virulence factor of many pathogenic *E. coli*, CNF1 deamidates GTPases leading to altered cytoskeletal rearrangements within the host target cell. This may be of key importance in the pathogenesis of UPEC bacteria as alterations in the neutrophil cytoskeleton may influence the neutrophil response. This

altered response is demonstrated in this study by the differences in levels and the amount of leukocytes seen in prostate tissues exposed to CNF1-positive bacteria as compared to CNF1-negative bacteria.

The model of disease used in this study is representative of many pathogenic processes caused by bacterial infection. While bacteria themselves can be the direct cause of pathology, one cannot discount the role of the host's inflammatory reaction. Many diseases have involvement by virulent organisms such as UPEC, but it is the immune response of the infected individual that leads to much of the pathology. There are many examples of this, such as pneumococcal pneumonia in which massive neutrophil influx into the lungs causes severe bystander injury and is the true source of much of the overt disease (90). There are also many diseases with no known pathogen as the etiological agent, and in these instances the inflammatory response is key in the disease process. Inflammatory bowel disease, interstitial cystitis, and abacterial prostatitis are excellent examples of this (27, 35, 48, 54, 57, 74).

By studying the role of a single virulence factor, we can determine its impact on the inflammatory process. This is a common strategy used in a wide variety of disease models, and has been employed to study individual virulence factors of pathogenic organisms. Urinary tract infection models have described the importance of P-fimbriae and Type-1 fimbriae of UPEC in stimulating an inflammatory response by the host (34, 94). The common trend found in these models is that each virulence factor stimulates the

inflammatory reaction, and may also assist the bacteria in colonization or avoiding phagocytosis (38). There is no one "master" virulence factor – instead the pathogen uses an assortment of virulence factors in concert.

The CNF1 mutants used in this study caused various levels of disease in test animals, revealing that by removing CNF1, one does not render the bacteria avirulent. This is not surprising, as UPEC strains carry a broad spectrum of virulence factors that would all contribute to the disease process (95). The decrease in inflammatory damage associated with the CNF1-negative mutant challenged animals is interesting, supporting a pro-inflammatory role for CNF1.

The exact role of CNF1 in urinary tract infections has yet to be fully determined, but previous investigations have revealed a broad spectrum of activity. CNF1 can induce cytokine release and vacuolation in epithelial cells (77), stimulate non-specific phagocytosis and inhibit specific receptor-mediated phagocytosis (32, 38), enhance the adhesion and oxidative burst of neutrophils (38) and decrease their transepithelial migration (37). The most significant effects of CNF1 are through actin cytoskeleton rearrangement. The toxin deamidates small GTPases to leave them constitutively active, causing the target cell to lose control of its cytoskeleton rearrangement and cytokinesis (13).

In the model used here, the impact of the neutrophil response was clearly altered by removing CNF1. The differences in neutrophil accumulation and ductal infiltration, coupled with the decrease in neutrophil-related pro-inflammatory cytokines suggest that CNF1 has a role in affecting the neutrophil response. It is this specific role that must be more closely examined. Questions regarding the specific effects of CNF1 on the neutrophil and the timing of these events should next be studied. For example, does a CNF1-induced increase in cytokines lead to an increased neutrophil response, or does an enhanced neutrophil recruitment precipitate a increased cytokine response? One direction of experimentation should be to examine the source of the inflammatory mediators, and the time course during the infection at which they are produced.

Further studies using this model to examine the role of CNF1 should involve the ductal epithelium of the prostate. The role of CNF1 should be examined with regards to its impact on the epithelium, not just the neutrophil. One cannot discount the importance of the epithelium in the infectious process of UPEC, and these cells may play a key role in the inflammatory process. Mucosal epithelium in other sites of the body, for example the gastrointestinal tract, can produce inflammatory mediators capable of recruiting inflammatory cells such as neutrophils (24). In the model presented here, the epithelial cells are the first to be exposed to the bacteria, and a pro-inflammatory response by the epithelium could be a cause of much of the inflammatory reaction seen.

Alterations to the epithelial cytoskeleton could affect the colonization and invasive capabilities of UPEC, and possibly the epithelial transmigration into the ducts by recruited neutrophils. Extensive in vitro studies similar to this have been carried out using gastrointestinal epithelial monolayers, and more recently have been carried out with urinary tract epithelium (40, 42). To further expand on the work presented here, in vitro studies similar to those carried out with gut and urinary epithelium should be performed using prostatic epithelial monolayers. While the prostate is part of the urinary system, its epithelial structure is different from that seen in the bladder or urethra. Because of this, pathogen attachment, neutrophil transmigration, and epithelial inflammatory responses may differ in the prostate from what is seen in the rest of the urinary system.

As we gain more knowledge about infectious and inflammatory diseases, it is becoming clear that rarely is it simply the pathogen that must be focused on. A disease that involves a virulent microorganism and a pathological inflammatory response must have both sides of the situation addressed. One can envision a combination of antimicrobial and anti-inflammatory regimens to combat such diseases and minimize host injury. By focusing on both aspects of the disease, one could minimize colonization and therefore decrease the pathogenicity of the infection while at the same time, anti-inflammatory treatments could prevent non-specific host bystander injury.

Bacterial prostatitis is a complex disease involving both bacterial and abacterial pathology. While damage can be due to the bacterial infection, the host's immune response is also involved. Here we show a role for CNF1 as a virulence factor that stimulates an inflammatory response in the prostate of a UPEC-infected host. By targeting CNF1 in prostatitis or other UPEC diseases, inflammatory complications may be alleviated.

C. Summary

Scientific research relies heavily on data produced in a synthetic laboratory setting. *In vitro* experiments allow for multiple samples, reproducibility, and techniques that reach a molecular level.

Unfortunately, *in vitro* research has an inherent flaw – in vitro results cannot be directly applied to the human system. The complexity of the body can cause unforeseen interference in the experimental procedure, leading to results not predicted by *in vi*tro studies.

Obviously, we cannot use human subjects to study previously untested scientific and medical research. The next best option is the use of animals for *in vivo* experiments. With this body of research, I have explored the potential uses for an animal model of disease, specifically prostatitis in a rat model. First, a novel method to simulate abacterial prostatitis was developed. This

was then used as a research tool to study factors of the inflammatory process. Pre-existing models were also manipulated in similar ways to reproduce a bacterial prostatitis model. This model can then be used for studying inflammation and infection in an *in vivo* system. Initial research using these models was done in this body of work, however, much remains to be investigated.

LITERATURE CITED

1. **Alexander, R. B., F. Brady, and S. Ponniah.** 1997. Autoimmune prostatitis: evidence of T cell reactivity with normal prostatic proteins. Urology **50:**893-899.

2. **Alexander, R. B., S. Ponniah, J. Hasday, and J. R. Hebel.** 1998. Elevated levels of proinflammatory cytokines in the semen of patients with chronic prostatitis/chronic pelvic pain syndrome. Urology **52:**744-749.

3. **Anderson, R. U., and C. Weller.** 1979. Prostatic secretion leukocyte studies in non-bacterial prostatitis (prostatosis). The Journal of Urology **121:**292-294.

4. **Andreu, A., A. E. Stapleton, C. Fennell, H. A. Lockman, M. Xercavins, F. Fernandez, and W. E. Stamm.** 1997. Urovirulence determinants in Escherichia coli strains causing prostatitis [published erratum appears in J Infect Dis 1997 Nov;176(5):1416]. J Infect Dis **176:**464-9.

5. **Baert, L., and E. Van Thillo.** 1994. Immunotherapy and prostatitis., p. 210-212. *In* W. Weidner, P. O. Madsen, and H. G. Shiefer (ed.), Prostatitis: etiopathology, diagnosis and therapy. Springer-Verlag, Berlin.

6. **Blacklock, N. J.** 1994. The anatomy of the prostate: relationship with prostatic infection., p. pp 32-40. *In* W. Weidner, P. O. Madsen, and H. G. Schiefer (ed.), Prostatitis: etiopathology, diagnosis, and therapy. Springer-Verlag, Berlin.

7. **Boag, A. H., and I. D. Young.** 1999. Pathology of acute and chronic prostate inflammation., p. 57-68. *In* J. C. Nickel (ed.), Textbook of Prostatitis. Isis Medical Media, Oxford, UK.

8. **Bradley, P. P., D. A. Priebat, R. D. Christensen, and G. Rothstein.** 1982. Measurement of cutaneous inflammaiton: estimation of neutrophil content with an enzyme marker. The Journal of Investigative Dermatology **78:**206-209.

9. **Brooks, S. J. D., J. M. Lyons, and A. I. Braude.** 1977. Immunization against retrograde pyelonephritis. III. Vaccination against chronic pyelonephritis due to *Escherichia coli*. The Journal of Infectious Diseases **136:**633-639.

10. **Bryan, R. L., J. Newman, A. Campbell, G. Fitzgerald, C. Kadow, and J. M. O'Brien.** 1991. Granulomatous prostatitis: a clinicopathological study. Histopathology **19:**453-457.

11. **Burnett, A. L., M. P. Macguire, S. L. Chamness, D. D. Ricker, M. Takeda, H. Lepor, and T. S. K. Chang.** 1995. Characterization and localization of nitric oxide sythase in the human prostate. Urology **45:**435-439.

12. **Capo, C., S. Meconi, M. V. Sanguedolce, N. Bardin, G. E. Flatau, P. Boquet, and M. J. L.** 1998. Effect of cytotoxic necrotizing factor-1 on actin cytoskeleton in human monocytes: Role in the regulation of integrin-dependent phagocytosis. Journal of Immunology **161:**4301-4308.

13. **Caprioli, A., G. Donelli, V. Falbo, L. Possenti, G. Roda, G. Roscetti, and F. M. Ruggeri.** 1984. A cell division active protein from Escherichia coli. Biochemistry and Biophysics Research Communications **118:**587-593.

14. **Cominelli, F., C. C. Nast, B. D. Clark, R. Schindler, R. Llerena, V. E. Eysselein, R. C. Thompson, and C. A. Dinarello.** 1990. Interleukin 1 (IL-1) gene expression, synthesis, and effect of specific IL-1 receptor blockade in rabbit immune complex colitis. Journal of Clinical Investigation **86:**972-980.

15. **Depiante-Depaoli, M., B. Pacheco-Rupil, S. Britos, and A. Casas.** 1984. Experimental autoimmune damage to rat male accessory glands. I. Transfer of autoimmune response by spleen cells. American Journal of Reproductive Immunology **5:**9-14.

16. **Dhundee, J., and A. G. Maciver.** 1991. An immunohistological study of granulomatous prostatitis. Histopathology **18:**435-441.

17. **Dilworth, J. P., D. E. Neal, E. N. Fussell, and J. A. Roberts.** 1990. Experimental prostatitis in nonhuman primates: I. Bacterial adherence in the urethra. The Prostate **17:**227-231.

18. **Diserio, G. P., and E. Nowotny.** 1998. Experimental autoimmune prostatitis: in vivo induction of the autoimmune response to lymphocytic soluble factors. Alterations at the endocrine metabolism level. American Journal of Reproductive Immunology **39:**226-234.

19. **Doble, A.** 1999. The diagnosis, aetiology and pathogenesis of chronic non-bacterial prostatitis., p. 129-137. *In* J. C. Nickel (ed.), Textbook of Prostatitis. Isis Medical Media, Oxford, UK.

20. **Domingue, G. J., and W. J. G. Hellstrom.** 1998. Prostatitis. Clinical Microbiology Reviews **11:**604-613.

21. **Donadio, A. C., and M. Depiante-Depaoli.** 1997. Inflammatory cells and MHC class II antigens expression in prostate during time-course experimental autoimmune prostatitis development. Clinical Immunology and Immunopathology **85:**158-165.

22. **Donovski, L.** 1989. Immunotherapy with Solco Urovac vaccine in chronic genitourinary infection. Khirurgiia **42:**28-30.

23. **Dumas, F., P. Eschwege, and S. Loric.** 1997. Acute bacterial prostatitis induces hematogenous dissemination of prostate epithelial cells. Clinical Chemistry **43:**2007-2008.

24. **Ernst, P. B., F. Song, G. R. Klimpel, H. Haeberle, K. B. Bamford, S. E. Crowe, G. Ye, and V. E. Reyes.** 1999. Regulation of the mucosal immune response. American Journal of Tropical Medicine and Hygiene **60:**2-9.

25. **Evans, D. T. P.** 1999. Medical management of chronic non-bacterial prostatitis., p. 293-309. *In* J. C. Nickel (ed.), Textbook of Prostatitis. Isis Medical Media, Oxford, UK.

26. **Falzano, L., C. Fiorentini, G. Donelli, E. Michel, C. Kocks, P. Cossart, L. Cabanie, E. Oswald, and P. Boquet.** 1993. Induction of phagocytic behaviour in human epithelial cells by *Escherichia coli* cytotoxic necrotizing factor type 1. Molecular Microbiology **9:**1247-1254.

27. **Felsen, D., S. Frye, L. A. Trimble, T. G. Bavendam, C. L. Parsons, Y. Sim, and J. E.D. Vaughan.** 1994. Inflammatory mediator profile in urine and bladder wash fluid of patients with interstitial cystitis. The Journal of Urology **152:**355-361.

28. **Fiorentini, C., G. Arancia, A. Caprioli, V. Falbo, F. M. Ruggeri, and G. Donelli.** 1988. Cytoskeletal changes induced in HEp-2 cells by the cytotoxic necrotizing factor of Escherichia coli. Toxicon **26:**1047-1046.

29. **Flatau, G. E., L. E., M. Gauthier, P. Chardin, S. Paris, C. Fiorentini, and P. Boquet.** 1997. Toxin-induced activation of the G protein p21 Rho by deamidation of glutamine. Nature **387:**729-733.

30. **Fong, L., C. L. Ruegg, D. Brockstedt, E. G. Engleman, and R. Laus.** 1997. Induction of tissue-specific autoimmune prostatitis with prostatic acid phosphatase immunization. The Journal of Immunology **159:**3113-3117.

31. **Gerhard, R., G. Schmidt, F. Hofman, and K. Aktories.** 1998. Activation of Rho GTPases by *Escherichia coli* cytotoxic necrotizing factor

1 increases intestinal permeability in Caco-2 cells. Infection and Immunity **66:**5125-5131.

32. **Gerhard, R., G. Schmidt, F. Hofmann, and K. Aktories.** 1998. Activation of Rho GTPases by *Escherichia coli* Cytotoxic Necrotizing Factor 1 increases intestinal permeability in Caco-2 cells. Infection and Immunity **66:**5125-5131.

33. **Gionchetti, P., M. Campieri, A. Belluzzi, E. Bertinelli, M. Ferretti, C. Brignola, G. Poggioli, M. Miglioli, and L. Barbara.** 1994. Mucosal concentrations of interleukin-1β, interleukin-6, interleukin-8, and tumor necrosis factor-α in pelvic ileal pouches. Digestive Diseases and Sciences **39:**1525-1531.

34. **Gunther IV, N. W., J. A. Snyder, V. Lockatell, I. Blomfield, D. E. Johnson, and H. L. T. Mobley.** 2002. Assessment of Virulence of Uropathogenic Escherichia coli Type 1 Fimbrial Mutants in Which the Invertible Element Is Phase-Locked On or Off. Infect. Immun. **70:**3344-3354.

35. **Higa, A., T. Eto, and Y. Nawa.** 1997. Evaluation of the role of neutrophils in the pathogenesis of acetic acid-induced colitis in mice. Scandinavian Journal of Gastroenterology **32:**564-568.

36. **Hingorani, A., E. Ascher, M. Scheinman, W. Yorkovich, P. DePippo, C. T. Ladoulis, and S. Salles-Cunha.** 1998. The effect of tumor necrosis factor binding protein and interleukin-1 receptor antagonist on the development of abdominal aortic aneurysms in a rat model. Journal of Vascular Surgery **28:**522-526.

37. **Hofman, P., G. Flatau, E. Selva, MGauthier, B. Rossi, and P. Boquet.** 1998. Escherichia coli cytotoxic necrotizing factor 1 (CNF-1) effaces microvilli and decreases transmigration of polymorphonuclear leukocytes in intestinal T84 epithelial cell monolayers. Infection & Immunity **66:**2494-2500.

38. **Hofman, P., G. L. Negrate, B. Mograbi, V. Hofman, P. Brest, A. Alliana-Schmid, G. Flatau, P. Boquet, and B. Rossi.** 2000. Escherichia coli cytotoxic necrotizing factor-1 (CNF-1) increases the

adherence to epithelia and the oxidative burst of human polymorphonuclear leukocytes but decreases bacteria phagocytosis. Journal of Leukocyte Biology **68:**522-528.

39. **Imai, H., M. Kabuto, M. Takita, and N. Kato.** 1998. Interleukin-1 receptor antagonist inhibits transient increase of plasma corticosterone in the initial phase of trimethylin-induced hippocampal necrosis. Neurotoxicology **19:**163-166.

40. **Island, M. D., X. Cui, B. Foxman, C. F. Marrs, W. E. Stamm, A. E. Stapleton, and J. W. Warren.** 1998. Cytotoxicity of Hemolytic, Cytotoxic Necrotizing Factor 1-Positive and -Negative Escherichia coli to Human T24 Bladder Cells. Infection and Immunity **66:**3384-3389.

41. **Island, M. D., X. Cui, and J. W. Warren.** 1999. Effect of Escherichia coli cytotoxic necrotizing factor 1 on repair of human bladder cell monolayers in vitro. Infect Immun **67:**3657-61.

42. **Island, M. D., X. Cui, and J. W. Warren.** 1999. Effect of Escherichia coli Cytotoxic Necrotizing Factor 1 on repair of human bladder cell monolayers in vitro. Infection and Immunity **67:**3657-3661.

43. **Kelsall, B., and W. Strober.** 1999. Gut-associated lymphoid tissue: antigen handling and T-lymphocyte responses., p. 293-318. *In* P. L. Ogra, J. Mestecky, M. E. Lamm, W. Strober, J. Bienenstock, and J. R. McGhee (ed.), Mucosal Immunology, 2 ed. Academic Press, San Diego.

44. **Khosravi-Far, R., P. A. Solski, G. J. Clark, M. S. Kinch, and D. C. J.** 1995. Activation of Rac1, RhoA, and Mitogen-Activated Protein Kinases is required for Ras transformation. Mol. Cell. Biol. **15:**6443-6453.

45. **Krieger, J. K., D. E. Riley, M. C. Roberts, and R. E. Berger.** 1996. Prokaryotic DNA sequences in patients with chronic idiopathic prostatitis. Journal of Clinical Microbiology **34:**3120-3128.

46. **Kumon, H.** 1999. Immunological aspects of prostatitis., p. 157-164. *In* J. C. Nickel (ed.), Textbook of Prostatitis. Isis Medical Media, Oxford, UK.

47. **Lang, C. H., J. Fan, R. Cooney, and T. C. Vary.** 1996. IL-1 receptor antagonist attenuates sepsis-induced alterations in the IGF system and protein synthesis. American Journal of Physiology **270:**E430-E437.

48. **Lang, M. D., J. C. Nickel, M. E. Olson, S. R. Howard, and H. Ceri.** 2000. Rat model of experimentally induced abacterial prostatitis., p. 201-6, Prostate, vol. 45.

49. **Lerm, M., G. Schmidt, and K. Aktories.** 2000. Bacterial protein toxins targeting Rho GTPases. FEMS Microbiology Letters **188:**1-6.

50. **Madsen, P. O., P. Drescher, and T. C. Gasser.** 1994. Classification, etiology, and diagnosis of prostatitis., p. 3-8. *In* Weidner, Madsen, and Schiefer (ed.), Prostatitis: etiopathology, diagnosis, and therapy. Springer-Verlag, Berlin.

51. **Meares, E. M.** 1991. Prostatitis. Medical Clinics of North America **75:**405-424.

52. **Mills, M., M. K.C., and O. B. A.D.** 2000. Cytotoxic Necrotizing Factor Type 1 of uropathogenic Escherichia coli kills cultured human

uroepithelial 5637 cells by an apoptotic mechanism. Infection and Immunity.

53. **Mitsumori, K., A. Terai, S. Yamamoto, S. Ishitoya, and O. Yoshida.** 1999. Virulence characteristics of *Escherichia coli* in acute bacterial prostatitis. The Journal of Infectious Diseases **180:**1378-1381.

54. **Mitsuyama, K., A. Toyonaga, E. Sasaki, K. Watanabe, H. Tateishi, T. Nishiyama, T. Saiki, H. Ikeda, O. Tsuruta, and K. Tanikawa.** 1994. IL-8 as an important chemoattractant for neutrophils in ulcerative colitis and Crohn's disease. Clinical and Experimental Immunology **96:**432-436.

55. **Morris, G. P., P. L. Beck, M. S. Herridge, W. T. Depew, M. R. Szewczuk, and J. L. Wallace.** 1989. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology **96:**795-803.

56. **Muntzig, J., G. Sufrin, and G. P. Murphy.** 1979. Prostatitis in the rat. Scandinavian Journal of Urology and Nephrology **13:**17-22.

57. **Mysorekar, I. U., M. A. Mulvey, S. J. Hultgren, and J. I. Gordon.** 2002. Molecular regulation of urothelial renewal and host defenses during infection with uropathogenic *Escherichia coli*. Journal of Biological Chemistry **277**.

58. **Naber, K. G.** 1999. Antibiotic treatment of chronic bacterial prostatitis., p. 285-292. *In* J. C. Nickel (ed.), Textbook of Prostatitis. Isis Medical Media, Oxford, UK.

59. **Nadler, R. B., P. A. Humphrey, D. S. Smith, W. J. Catalona, and T. L. Ratliffe.** 1995. Effect of inflammation and benign prostatic hyperplasia on elevated serum prostate specific antigen levels. The Journal of Urology **154:**407-413.

60. **Nadler, R. B., A. E. Koch, E. A. Calhoun, P. L. Campbell, D. L. Pruden, C. L. Bennett, P. R. Yarnold, and A. J. Schaeffer.** 2000. IL-1β and TNF-α in prostatic secretions are indicators in the evaluation of men with chronic prostatitis. The Journal of Urology **164:**214-218.

61. **Naslund, M. J., J. D. Strandberg, and D. S. Coffey.** 1988. The role of androgens and estrogens in the pathogenesis of experimental nonbacterial prostatitis. The Journal of Urology **140:**1049-1053.

62. **Neal, D. E.** 1999. Treatment of acute prostatitis., p. 279-284. *In* J. C. Nickel (ed.), Textbook of Prostatitis. Isis Medical Media, Oxford, UK.

63. **Nickel, J. C., and J. W. Costerton.** 1992. Coagulase-negative *Staphylococcus* in chronic prostatitis. The Journal of Urology **147:**398-401.

64. **Nickel, J. C., J. W. Costerton, R. J. McLean, and M. Olson.** 1994. Bacterial biofilms: influence on the pathogenesis, diagnosis and treatment of urinary tract infections. Journal of Antimicrobial Chemotherapy **33:**31-41.

65. **Nickel, J. C., J. Downey, I. D. Young, and A. H. Boag.** 1999. Asymptomatic inflammation and/or infection in benign prostatic hyperplasia. Journal of Urology **161:**30A.

66. **Nickel, J. C., M. E. Olson, A. Barabas, H. Benediktsson, M. K. Dasgupta, and J. W. Costerton.** 1990. Pathogenesis of chronic bacterial prostatitis in an animal model. British Journal of Urology **66:**47-54.

67. **Nickel, J. C., M. E. Olson, and J. W. Costerton.** 1991. Rat model of experimental bacterial prostatitis. Infection **19:**S126-S130.

68. **Niku, S. D., P. C. Stein, H. C. Scherz, and C. L. Parsons.** 1994. A new method for cytodestruction of bladder epithelium using protamine sulfate and urea. The Journal of Urology **152:**1025-1028.

69. **Nishimura, T., H. Abe, H. Ito, K. Ikeda, F. Oka, and M. Yamamoto.** 1998. IL-1ra versus IL-1 levels in prostatic fluid from prostatitis patients. Urologia Internationalis **60:**92-96.

70. **O'Dea, M. J., D. B. Huntington, and L. F. Greene.** 1977. Non-specific granulomatous prostatitis. Journal of Urology **118:**58-60.

71. **Oke, K. E.** 1996. Galectins in the prostate. The University of Calgary, Calgary.

72. **Parsons, C. L.** 1994. The therapeutic role of sulfated polysaccharides in the urinary bladder. Urologic Clinics of North America **21:**93-99.

73. **Parsons, C. L., D. Boychuk, S. Jones, R. Hurst, and H. Callahan.** 1990. Bladder surface glycosaminoglycans: an epithelial permeability barrier. The Journal of Urology **143:**139-142.

74. **Parsons, C. L., J. D. Lilly, and P. Stein.** 1991. Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis). The Journal of Urology **145:**732-735.

75. **Persson, B.-E., and G. Ronquist.** 1996. Evidence for a mechanistic association between nonbacterial prostatitis and levels of urate and creatinine in expressed prostatic secretion. The Journal of Urology **155:**958-960.

76. **Prendergast, G. C., K.-F. R., P. A. Solski, K. H., L. P. F., and D. C. J.** 1995. Critical role for Rho in cell transformation by oncogenic Ras. Oncogene **10:**2289-2296.

77. **Reyrat, J. M., V. Pelicic, E. Papini, C. Montecucco, R. Rappuoli, and J. L. Telford.** 1999. Toward deciphering the Helicobacter pylori cytotoxin. Molecular Microbiology **34:**197-204.

78. **Rippere-Lampe, K. E., M. D. Lang, H. Ceri, M. E. Olson, H. A. Lockman, and A. D. O'Brien.** 2001. Cytotoxic Necrotizing Factor Type 1-positive *Eschericia coli* causes increased inflammation and tissue damage to the prostate in a rat prostatitis model. Infection and Immunity **69:**6515-6519.

79. **Rippere-Lampe, K. E., A. D. O'Brien, R. Conran, and H. A. Lockman.** 2001. Mutation of the gene encoding Cytotoxic Necrotizing Factor Type 1 (*cnf*1) attenuates the virulence of uropathogenic *Eschericia coli*. Infection and Immunity **69:**3954-3964.

80. **Roberts, R. O., M. M. Lieber, D. G. Bostwick, and S. J. Jacobsen.** 1997. A review of clinical and pathological prostatitis syndromes. Urology **49:**809-821.

81. **Ruggeri, F. M., C. Fiorentini, A. Caprioli, G. Arancia, V. Falbo, and D. G.** 1986. HEp-2 cells multinucleation induced by an E. coli cytotoxic factor. IRCS Med. Sci. **14:**833-834.

82. **Schaeffer, A. J.** 1994. Etiology, pathogenesis, and inflammatory reactions in chronic bacterial prostatitis., p. 152-157. *In* W. Wiedner, P. O. Madsen, and H. G. Schiefer (ed.), Prostatitis: etiopathology, diagnosis and therapy. Springer-Verlag, Berlin.

83. **Schmidt, G., P. P. Sehr, M. M. Wilm, J. Selzer, M. Mann, and K. Aktories.** 1997. Gln 63 of Rho is deamidated by Escherichia coli cytotoxic necrotizing factor-1. Nature **387:**725-729.

84. **Seethalakshmi, L., R. S. Bala, R. K. Malhotra, T. Austin-Ritchie, C. Miller-Graziano, M. Menon, and J. Luber-Narod.** 1996. 17b-estradiol induced prostatitis in the rat is an autoimmune disease. The Journal of Urology **156:**1838-1842.

85. **Shortliffe, L. M., R. G. Sellers, and J. Schachter.** 1992. The characterization of bacterial prostatitis: search for an etiology. The Journal of Urology **148:**1461-1466.

86. **Slomiany, A., A. Takagi, and B. L. Slomiany.** 1985. The synthesis and secretion of gastric mucus glycoprotein by mucosal cells cultured in the presence of ethanol. Archives of Biochemistry & Biophysics **236:**654-661.

87. **Sugihara, A., O. Maeda, M. Tsuji, T. Tsujimura, Y. Nakata, H. Akedo, T. Kotake, and N. Terada.** 1998. Expression of cytokines enhancing the osteoclast activity, and parathyroid hormone-related protein in prostatic cancers before and after endocrine therapy: an immunohistochemical study. Oncology Reports **5:**1389-1394.

88. **Taguchi, O., A. Kojima, and Y. Nishizuka.** 1985. Experimental autoimmune prostatitis after neonatal thymectomy in the mouse. Clinical and Experimental Immunology **60:**123-129.

89. **Terai, A., S. Ishitoya, K. Mitsumori, and O. Ogawa.** 2000. Molecular epidemiological evidence for ascending urethral infection in acute bacterial prostatitis. Jouurnal of Urology **164:**1945-1947.

90. **Wagner, J. G., and R. A. Roth.** 2000. Neutrophil migration mechanisms, with an emphasis on the pulmonary vasculature. Pharmacological Reviews **52:**349-374.

91. **Wallace, J. L., C. M. Keenan, D. Gale, and T. S. Shoupe.** 1992. Exacerbation of experimental colitis by nonsteroidal anti-inflammatory drugs is not related to elevated leukotriene B_4 synthesis. Gastroenterology **102:**18-27.

92. **Weidner, W., P. O. Madsen, and H. G. Schiefer.** 1994. Prostatitis: etiopathology, diagnosis, and therapy. Springer-Verlag, Berlin.

93. **Weiss, S. G., and T. D. Moon.** 1999. α-Blocker therapy., p. 319-327. *In* J. C. Nickel (ed.), Textbook of Prostatitis. Isis Medical Media, Oxford, UK.

94. **Wullt, B., G. Bergsten, H. Connell, P. Rollano, N. Gebratsedik, L. Hang, and C. Svanborg.** 2001. P-fimbriae trigger mucosal responses to Escherichia coli in the human urinary tract. Cellular Microbiology **3:**255-264.

95. **Yamamoto, S., T. Tsukamoto, A. Terai, H. Kurazono, Y. Takeda, and O. Yoshida.** 1995. Distribution of virulence factors in Escherichia coli isolated from urine of cystitis patients. Microbiol Immunol **39:**401-4.

THE UNIVERSITY OF CALGARY

The Inflammatory Process in Rat Models of Prostatitis.

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF DOCTOR OF PHILOSOPHY

CALGARY, ALBERTA

APRIL, 2005