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Inflammation

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| <b>14. ABSTRACT</b><br>With this proposal, we seek to determine the mechanisms for epithelial proliferation in response to inflammation, a process termed "reactive hyperplasia". The purpose of this report is to evaluate the first year of research on this project. We found that interleukin signaling is critical to the hyperplastic response of the prostate, and that proliferation is driven in the epithelium of the prostate by phosphoinositide-dependent action, while stromal proliferation appears dependent on Jak-STAT signaling. We expanded this project in response to a startling discovery: a myriad of inflammatory mediators are expressed at high levels during organogenesis of the prostate, a process that, like reactive hyperplasia, is characterized by rapid epithelial proliferation. We found the interleukin-1 signaling is critical to epithelial proliferation during organogenesis. Future research will determine the mechanisms of IL-1 action in development and reactive hyperplasia in the prostate. |                         |   |   |  |   |
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## INTRODUCTION

**Background:** Among the variety of genetic and environmental factors involved in the development of prostate cancer, chronic prostatic inflammation is believed to play a central role. Chronic inflammation is thought to create a microenvironment of sustained cell proliferation and activated stroma in which an abundance of inflammatory mediators, growth factors, and DNA damaging agents may initiate malignant transformation. Chronic inflammation is typically associated with dysplastic changes including nuclear polymorphism, cytoplasmic basophilia, and reactive epithelial hyperplasia. Interleukins (IL) 1 $\beta$  and 6, tumor necrosis factor (TNF), and prostanoid release all play a role in prostatic inflammation and have also been implicated as growth promoters in prostate cancer. In addition, proliferative inflammatory atrophy (PIA), a histologic lesion characterized by proliferating epithelial cells and activated inflammatory cells, is often found in association with prostatic intra-epithelial neoplasia (PIN) and prostate cancer and has been postulated to represent a pre-malignant lesion. These findings have prompted the hypothesis that chronic inflammation is involved in the genesis and/or progression of prostate cancer.

However, substantiation of a postulated causal relationship between inflammation and cancer in the prostate requires supporting evidence for inflammation-induced anomalies in growth regulation that could contribute to cancer development, studies that are thus far lacking. This proposal addresses that deficiency by using a combination of in vitro and in vivo approaches to examine and elucidate the effect of inflammatory cytokines and acute/chronic inflammation on the phosphoinositide-3 kinase (PI<sub>3</sub>K) pathway.

The PI<sub>3</sub>K/ Akt/ PTEN pathway is critical to cell cycle progression and has long been an interest to cancer researchers due to the frequent finding of PI<sub>3</sub>K induction, Akt phosphorylation and PTEN suppression in cancer. The PI<sub>3</sub>K cascade is induced by inflammation and inflammatory cytokines such as interleukins, and our preliminary data indicate that inflammatory cytokines induce PI<sub>3</sub>K activation, Akt activation, PTEN suppression and proliferation in cell culture. In addition, we have preliminary data showing that reactive hyperplasia and dysplasia in a recently described model of chronic prostatic inflammation is associated with PTEN suppression and Akt activation.

**Hypothesis:** Inflammation induces prostatic epithelial proliferation in a PI<sub>3</sub>K cascade-dependent fashion.

**Specific Aims:** We will: 1) To determine the effect of inflammatory cytokines on PI<sub>3</sub>K activation and cell proliferation in vitro; 2) To determine the effect of acute prostate inflammation on PI<sub>3</sub>K activation and epithelial proliferation in vivo; 3) To determine the role of the PI<sub>3</sub>K cascade in reactive hyperplasia and dysplasia associated with bacterial-induced chronic prostatic inflammation.

**Study Design:** In Aim 1, we will treat prostate epithelial cell lines with IL-1 $\beta$ , IL-6, TNF and measure PI<sub>3</sub>K activation by PIP<sub>3</sub> concentration and Akt activation, and

quantitate the effects on cell proliferation. We will use PI<sub>3</sub>K inhibitors to test the dependence of these responses on the PI<sub>3</sub>K cascade and verify this as necessary by siRNA-induced knockdown of PI<sub>3</sub>K and overexpression of PTEN. In aim 2, we will instill LPS, inflammatory cytokines, or PBS (control) into mouse prostates via urethral catheter and correlate the resulting inflammatory and proliferative response with PI<sub>3</sub>K cascade induction. We will evaluate inflammatory response by microscopic analysis, inflammatory mediator expression and release, PI<sub>3</sub>K activation by PIP<sub>3</sub> concentration and Akt activation, and cell proliferation via microscopy and marker staining. We will determine the PI<sub>3</sub>K dependence of the proliferative response with PI<sub>3</sub>K inhibitors and Akt and PTEN-deficient mice. In aim 3, we will use a recently characterized model of bacterial-induced chronic inflammation to comprehensively examine changes in PI<sub>3</sub>K activation, Akt phosphorylation and PTEN expression during the development of hyperplasia and reactive dysplasia. We will examine the dependence of these responses on the PI<sub>3</sub>K cascade using PTEN and Akt-deficient animals.

**Relevance:** Rigorous investigation of the molecular mechanisms connecting prostatic inflammation to effects on epithelial proliferation will provide new and critically important insights into the way chronic prostatic inflammation may promote tumorigenesis and cancer progression. The logical extension of this work includes prediction of prostate cancer risk based on molecular screening and specific molecular intervention for the treatment of prostatic inflammation. The recent development of PI<sub>3</sub>K isoform-specific inhibitors has heightened interest in this cascade as a potential therapeutic target and we expect the studies proposed here to provide a compelling rationale for examining their use in prostate cancer prevention as well as in slowing prostate cancer progression.

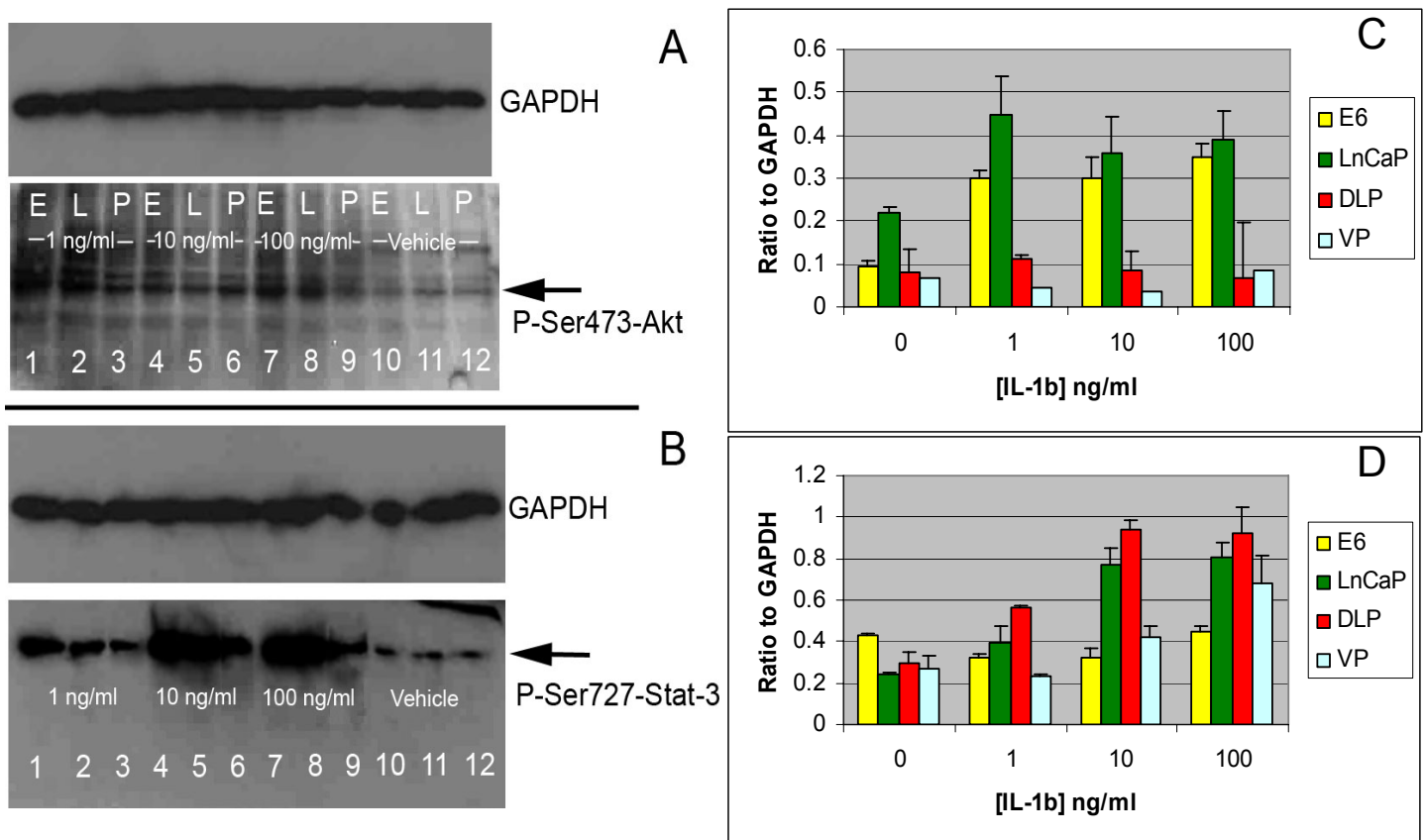
## **BODY**

In this section, I present data I have obtained with the funding from this proposal. I have completed the work expected by the timeline described in the statement of work (SOW). However, in my investigations, and under funding from this award, I came across a startling finding: that so-called “inflammatory mediators” are expressed and high levels during normal organogenesis of the prostate and play a key role in prostate development. I present data from these findings in addition to those outlined in the original proposal.

This section is divided in two parts: that which corresponds to tasks outlined in the statement of work for this proposal, and that which is related to the theme of the research project, but not clearly outlined in the statement of work.

**Task 1: [0-8 months] To determine the effect of LPS and inflammatory cytokines on PI<sub>3</sub>K activation, and cell proliferation of prostatic epithelial cells in vitro.**

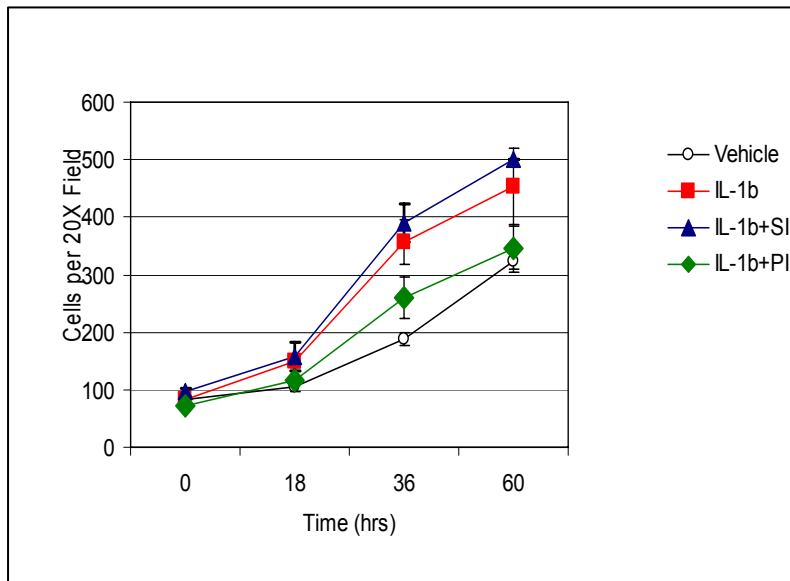
**A.** Do acute inflammatory signals induce the PI3K cascade in prostatic epithelial cells and prostate cancer cell lines? [0-6 months] We treated cultured prostate epithelial cells with 1, 10, or 100 ng/ml IL-1b or vehicle (0.2% BSA) for 4 hours and assessed activation of the PI3K pathway via phosphorylation of the key serine residue for activation, serine 473, with phospho-specific antibody. Two cancer cell lines (LNCaP and PC3) and one primary immortalized prostatic epithelial cell line (E6) were analyzed, along with three prostate stromal lines cultured in our laboratory from p16 (Ink4A) conditional (Fsp-driven) knockout mice. We found that all three epithelial lines, including cancer lines, responded to IL-1 induction by Akt activation. **[Figure 1]** Stromal lines did not show induction of PI3K signaling, but did respond to IL-1 stimulation by induction of Jak-STAT signaling, as evidenced by phosphorylation of serine 727 in STAT-3. These data suggest distinct signaling differences between the epithelial and stromal compartments of the prostate in response to IL-1 stimulation. The bacterial endotoxin lipopolysaccharide (LPS) did not induce signaling directly in our experiments.



**Figure 1.** IL-1 stimulation induces PI3K activity in epithelial cell lines, and Jak-STAT activity in stromal lines. **In panel A**, three epithelial lines were evaluated: E (E6 transformed human epithelial cells); L (LNCaP androgen-sensitive prostate cancer cells); and P (PC-3, androgen-insensitive prostate cancer cells). All three responded to IL-1 stimulation (concentrations indicated) with an increase in Akt phosphorylation. **In Panel B**, three mouse stromal cell lines were evaluated for IL-1 response at the concentrations indicated by STAT-3 phosphorylation. Quantified data for Akt activity are shown in **panel C**, with E6 and LNCaP representing epithelial cell lines, and cells cultured from the stroma of dorsolateral lobe (DLP) or ventral lobe (VP) of mouse prostates representing stromal lines. **Panel D** depicts quantified data from P-STAT-3 western blots analyzing these same cell lines. IL-1 treatment induces PI3K signaling in epithelial cells, but Jak-STAT signaling in stromal cells. All data represent the average of 3 experiments.

**B. Does treatment of prostatic epithelial cells and prostate cancer cell lines with inflammatory signals induce cell proliferation in a PI3K cascade-dependent fashion? [0-8 months]**

We treated epithelial cells with several inflammatory mediators, including IL-1a, IL-1b, IL-6, and TNF, and PGE2. All of these agents were able to induce cell proliferation in serum-free cell culture of prostate epithelial cells E6, E7, LNCaP and PC3. We further analyzed IL-1-induced growth for its dependence on Akt signaling, and found that pharmacological inhibition of PI3K attenuated this proliferative response. [Figure 2] In contrast, pharmacological inhibition of Jak-STAT signaling did not reduce IL-1-induced proliferation. Experiments are underway to determine the effect of signaling inhibition on stromal cell lines.



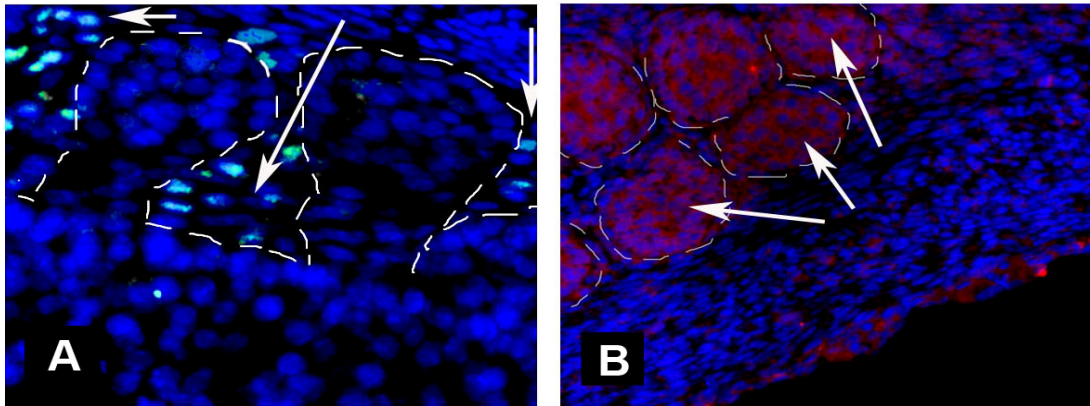
**Figure 2.** IL-1b treatment of E6-transfected prostate epithelial cells causes induced growth, as indicated by number of cells visible in a 20x field. Data reflect 15 determinations of each cultured set of cells for each data point, and 4 repetitions of each experiment. (n=4) Inhibition of PI3K signaling by a pharmacological PI3K inhibitor (PI, LY 294002-10  $\mu$ M; reported IC50=20  $\mu$ M) attenuated induced proliferation. However, inhibition of Jak-STAT signaling (with JakI-1, SI, 100nM; reported IC50=15 nM) did not attenuate the growth response)

**Task 2: [6-18 months] To determine the effect of acute inflammatory stimulation on PI<sub>3</sub>K activation, epithelial proliferation, and inflammatory response in mouse prostate.**

**A. Does instillation of acute inflammatory signals induce the PI<sub>3</sub>K cascade in the prostate epithelium? [6-12 months].**

We modified our original methodology written in the proposal due to the inconsistent results we obtained with the original protocol. In this study, we used developing mouse prostate as a model for prostatic epithelial growth in response to inflammatory signals. We treated embryonic day 16 mouse urogenital sinuses with IL-1a, IL-1b, IL-6, and IL-8, all of which had the capacity to induce epithelial proliferation similar to reactive hyperplasia during inflammation of adult prostates. We found that this treatment induced both Jak-STAT signaling as indicated by phosphorylated STAT-3, and PI3K signaling as indicated by phosphorylated Akt. However, STAT-3 induction is specific to the stromal compartment, while Akt activation is restricted to the epithelium (**Figure 3**). These data indicate that the

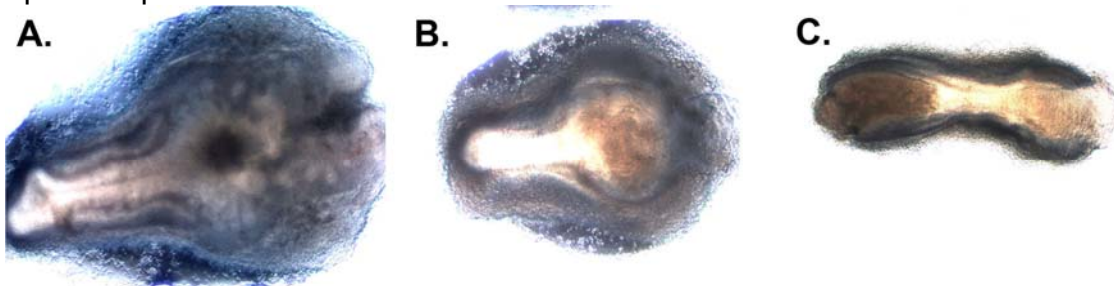
prostatic epithelium does respond to inflammatory signals by activation of PI3K signaling.



**Figure 3.** IL-1 induced Jak-STAT signaling in the mesenchymal cells of organ cultures as detected by phosphorylation of STAT-3 [D, cyan nuclei / green cytoplasm]. In contrast to isolated epithelial cell culture, IL-1 treatment induced PI3K signaling in the epithelium of organ cultures as measured by phosphorylated Akt [E, red cytoplasm].

**B.** Does acute exposure of the prostate epithelium to inflammatory signals induce inflammation and reactive proliferation in a PI3K cascade-dependent fashion? [10-18 months]

Using our ex vivo organ culture model to assess the function of inflammatory mediators on prostate epithelial proliferation and growth, we found that interleukin-induced epithelial growth is dependent upon both the PI3K cascade, as well as the Jak-STAT pathway. In organs treated with IL-1 $\alpha$ , co-treatment with Jak-STAT inhibitors completely attenuated both epithelial and stromal proliferation and growth, while treatment with Akt inhibitors inhibited only epithelial growth. (**Figure 4.**) These data indicate that while the PI3K-Akt pathway is involved in inflammatory mediator-induced growth, this effect is dependent upon stromal induction of Jak-STAT signaling, suggesting an paracrine epithelial-stromal interaction of interleukin signaling, and a secondary factor produced by stromal response to interleukins that signals back to the epithelium, resulting in epithelial proliferation.



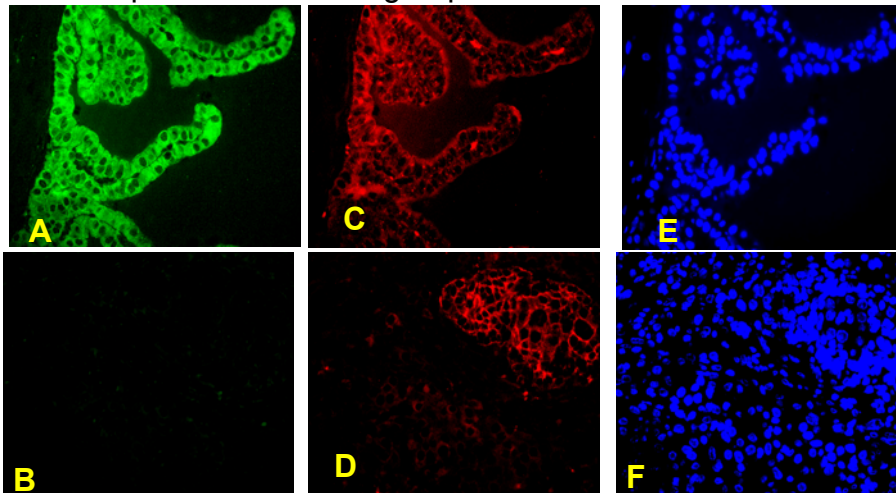
**Figure 4.** IL-1-induced expansion involves Jak-STAT signaling in the mesenchyme/stroma, and PI3K signaling in the epithelium. Inhibition of PI3K results in a loss of IL-1-induced epithelial expansion [B] relative to IL-1 alone [A], inhibition of Jak results is a full attenuation of both stromal and epithelial expansion [C].



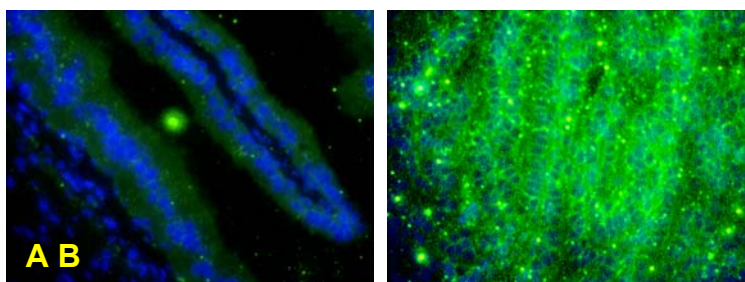
**AIM 3: [8-24 months] To determine the effect of the PI<sub>3</sub>K cascade on reactive hyperplasia in a previously characterized mouse model of chronic bacterial prostatitis.**

**A.** Is epithelial PI<sub>3</sub>K cascade activated in a novel model of chronic prostatic inflammation? [8-18 months]

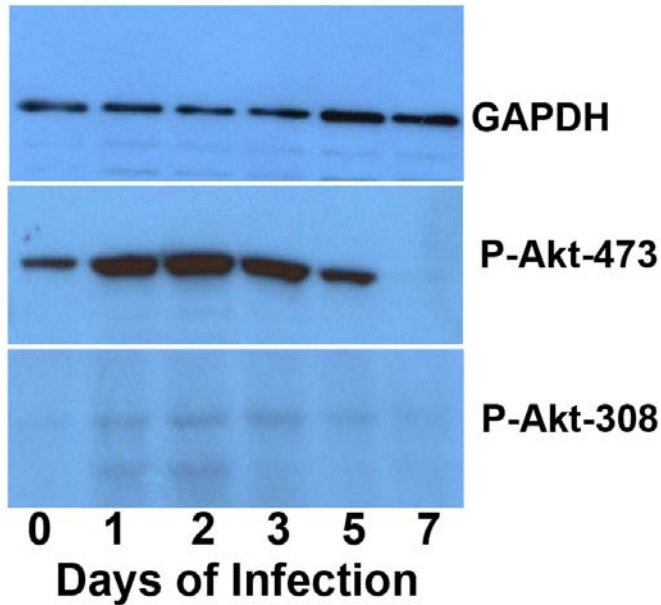
Reactive hyperplasia in chronically inflamed mouse prostates exhibits loss of PTEN expression and increased Akt phosphorylation. Areas of chronic inflammation and reactive hyperplasia in mice 26 weeks after inoculation were associated with a loss of PTEN expression, similar to what is observed in prostate cancer. **Figure 5** illustrates that PTEN staining (green) is ubiquitous in normal prostates (top) but is absent in chronically inflamed prostates (bottom). Pan-CK staining was performed to confirm epithelial identity of the cells. This result was consistently observed in a comparison of 6 infected and 6 control mice, with the degree of PTEN loss apparently correlating with the severity of inflammation. In addition, **Figure 6** illustrates that areas of chronic inflammation (right) exhibited a corresponding increase in Akt phosphorylation green compared to normal prostates (left). These data show not only that there is an increase in positive staining for P-Akt in inflamed prostates, but also in nuclear localization as the Hoechst-stained nuclei (blue) are co-localized with P-Akt, resulting in a cyan color seen in inflamed prostates (right). These data were repeatable across all six prostates in each group.



**Figure 5.** Normal epithelium [top: A,C,E] and chronically inflamed epithelium [bottom: B,D,F] probed for PTEN [A,B], Pan-CK [C,D], and Hoechst nuclei staining [E,F]. This figure shows that normal prostates exhibit strong staining for PTEN (A), but inflamed prostates do not exhibit positive PTEN expression (B).



**Figure 6.** Normal epithelium [A] and chronically inflamed epithelium [B] probed for phosphorylated Akt (green) and Hoechst nuclei staining (blue). Staining for P-Akt appears stronger in the epithelium of inflamed tissue (B) than normal prostate (A), and nuclear localization is present, as indicated by cyan-colored nuclei in inflamed prostate (B) that appears absent in normal tissue (A).



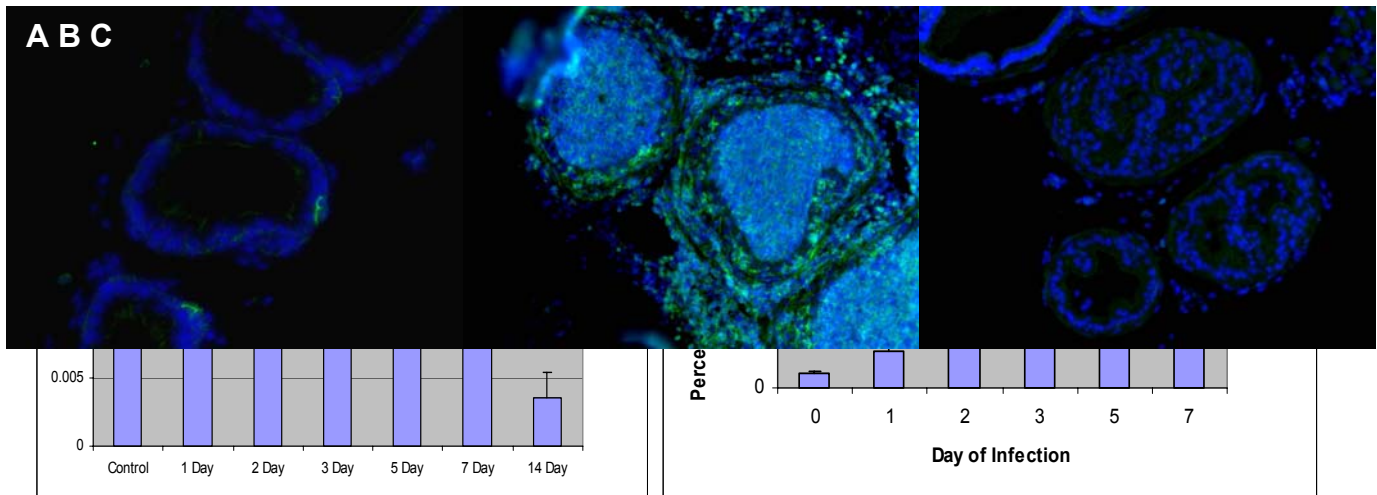
**Figure 7.** C57 wild-type mice were infected with *E. coli* for 1 to 7 days. Protein preparations from the prostates were analyzed by SDS-PAGE and immunoblotted for phosphorylated Akt on residues 473 and 308. Abundant phosphorylation on residue 473 indicates activation of the PI3K pathway during acute inflammation of the prostate. (n=4) Slight activation at residue 308 also is suggestive of activation, though is much less pronounced.

- B.** Is chronic prostatic inflammation associated with PI<sub>3</sub>K-dependent tissue damage and injury repair mechanisms and hyperplasia? [12-24 months]  
**C.** Does chronic prostatic inflammation induce reactive epithelial hyperplasia in a PI<sub>3</sub>K cascade-dependent fashion? [12-24 months]

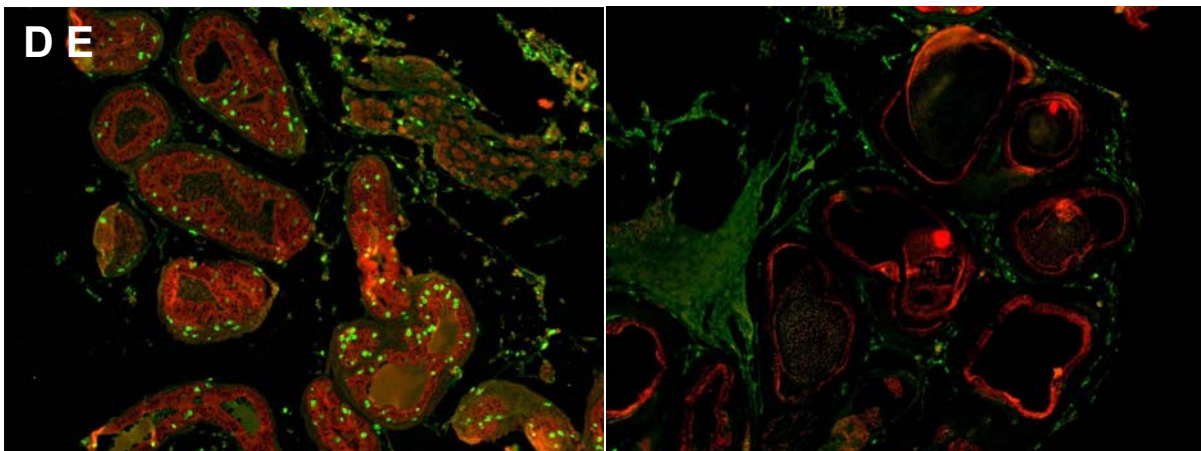
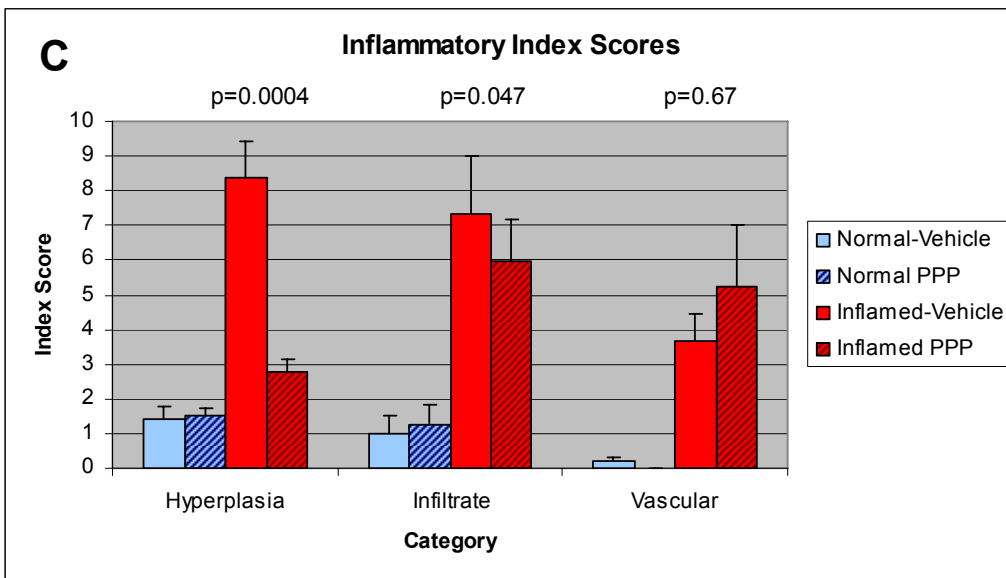
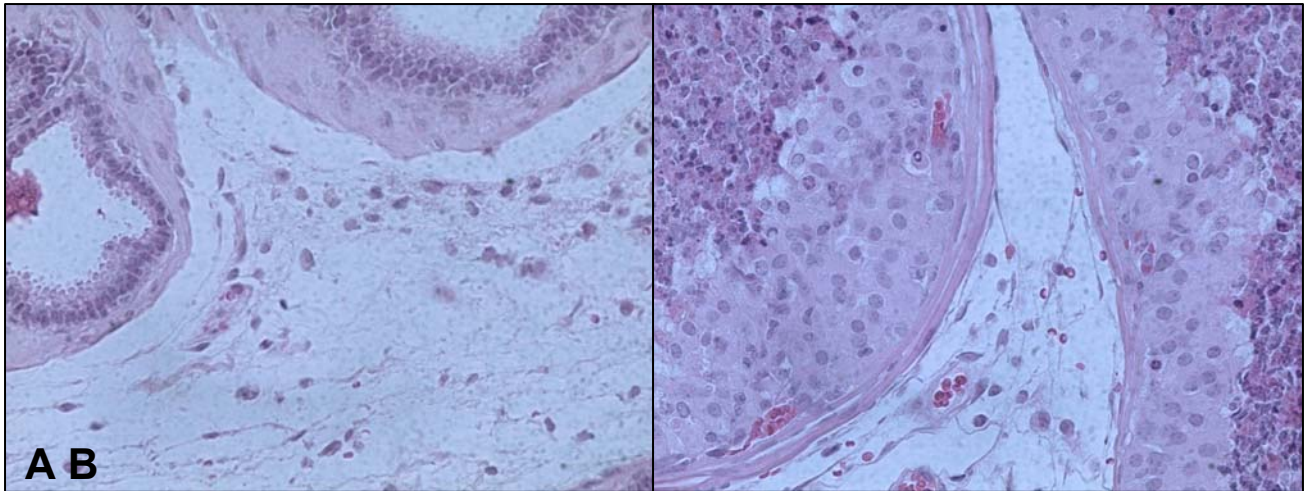
Concurrent studies to this proposal indicated that PI3K activation in response to inflammation was dependent on insulin-like growth factor signaling (please see “Experiments not detailed in the statement of work or specific aims” section following). Furthermore, our ex vivo organ culture model demonstrated that epithelial growth and proliferation in response to inflammatory signals is dependent upon IGF signaling. Because of this, and due to difficulties in in vivo use of PI3K inhibitors, we used the IGF signaling inhibitor picropodophyllin (PPP, 20 mg/kg/12 hours) to complete these studies. Based on previously published reports, we optimized a model of pharmacological IGF signaling inhibition using picropodophyllin (PPP). Previous reports indicate PPP effective at inhibiting IGF signaling in vivo and blocking the hyperplastic response in vessels. Our first attempts using this drug resulted in significant toxicity due to using a 90% DMSO solution in already sick animals. We have modified this to a 75% corn oil solution (25% DMSO) and reduced the total volume of the vehicle while still maintaining concentration of the drug. The result was effective inhibition of IGF signaling with no significant signs of toxicity. 20 mg/kg/12 hr was found to be optimal for IGF inhibition based on phospho-tyrosine IGFR staining. **(Figure 9)** These results demonstrate that our protocol for IGF inhibition with pharmacological inhibitors results in adequate IGF signaling inhibition to study the causal relationship between inflammation, IGF-1-Akt, and resulting reactive hyperplasia. To test whether prostatic inflammation induces PI3K/Akt/IGF-dependent hyperplasia, we inflamed animals by *E. coli* instillation and concurrently treated them with PPP or vehicle as described for three days. Mice treated with PPP exhibited significantly

less proliferation and hyperplasia than vehicle treated animals. Tissue damage remained similar between the groups. Inflammatory infiltrate and edema was also similar between the groups. **(Figure 10.)** These data indicate that IGF-1/ PI3K/ Akt signaling does not play a critical role in the inflammatory response or tissue damage caused by inflammation, but is critical to the proliferative repair mechanisms and hyperplasia that result from chronic inflammation and tissue damage.

**Figure 9** While normal prostate (A) shows limited active IGF1R1 (green/cyan), inflammation (3 days) substantially enhances activation (B). Inflamed prostates from mice concurrently treated with PPP (C) show substantially reduced IGF1R activation, indicating sufficient inhibition of IGF signaling.



**Figure 10.** Mice were infected for 3 days in presence **[A]** or absence **[B]** of PPP, an IGF signaling inhibitor. Inhibition of Akt signaling by inhibiting its primary inducer, IGF, attenuated inflammation-induced hyperplasia. Tissue damage did not appear to be affected by IGF and Akt inhibition. Quantified data from 6 animals in each group are displayed in **[C]**. P values are ANOVA calculations of inflamed vehicle vs. PPP. Animals were also treated with BrdU 1 hour prior to sacrifice. IHC staining for BrdU label reveals substantially induced epithelial proliferation during inflammation in vehicle-treated tissues **[D]**; this is completely attenuated by PPP **[E]**.





## Experiments not detailed in the statement of work or specific aims:

**A.** The pro-proliferative role of inflammatory cytokines in conditions of inflammation and malignancy prompted us to examine whether these cytokines play a corresponding role in the promoting normal growth during development. Using a custom cytokine gene array representing 128 cytokines and related proteins to screen for expression in the developing, adult and inflamed prostate, we found eight interleukins among the regulated genes which demonstrated higher expression in the E16 and P1 prostate than in the adult. All eight showed a significant increase in expression in a mouse model of acute bacterial infection [Table 1]. Fourteen other factors detected on the array as showing increased expression in the developing prostate and twelve exhibited increased expression in acute inflammation. Of the remaining 106 factors that either did not show increased expression in the developing prostate or were undetected by the array, only four exhibited induced expression in acute inflammation. This overview suggests that selective expression of inflammatory cytokines occurs in the developing prostate and that many of these same factors are re-expressed in the inflamed adult prostate.

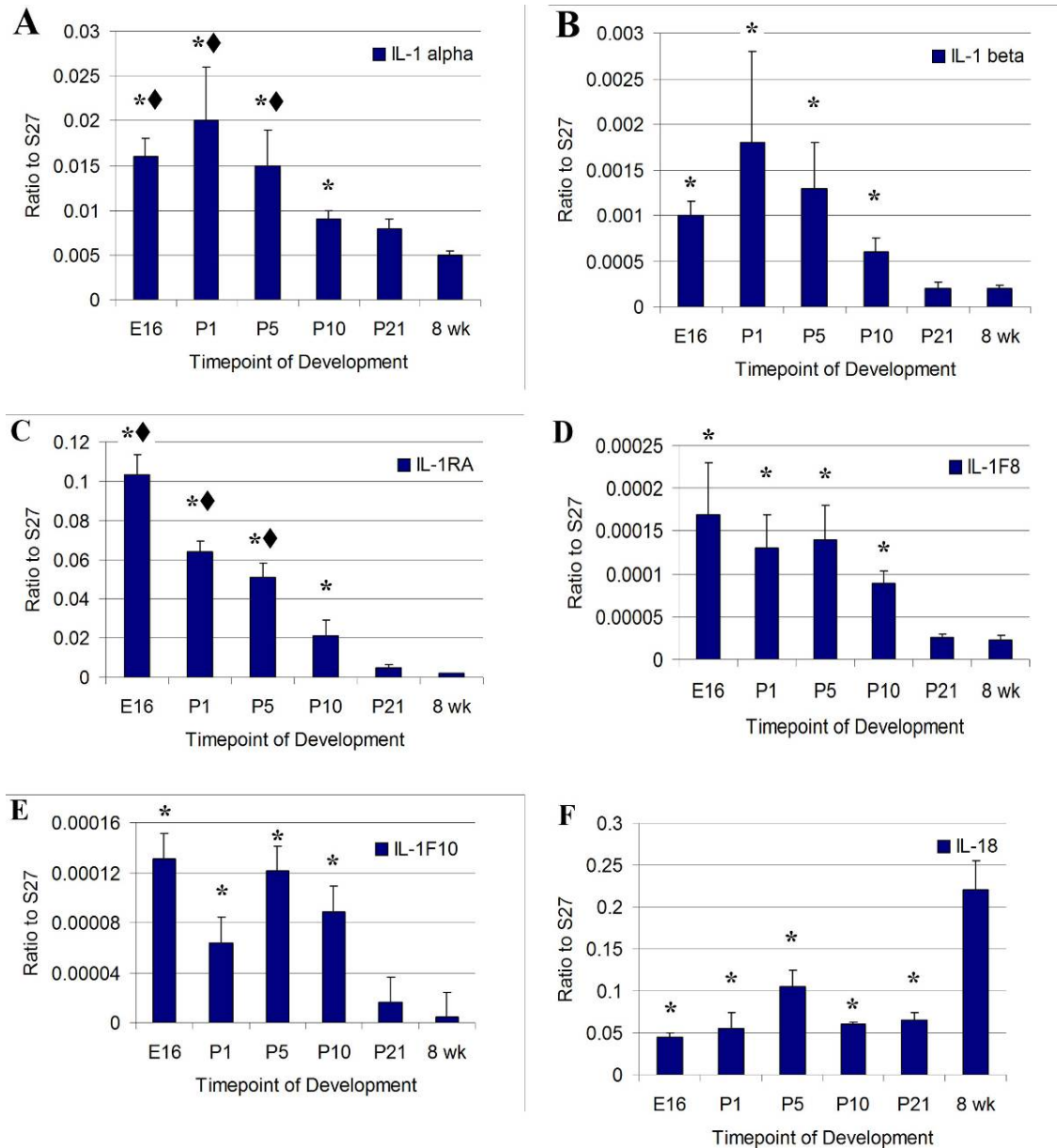
| Inflammatory Mediator                            | Fold change: E16/Adult | Fold change: P1/Adult | Fold change: Infl/Normal |
|--|------------------------|-----------------------|--------------------------|
| <i>Interleukin 1<math>\alpha</math></i>          | 8.3                    | 7.4                   | 6.6                      |
| <i>Interleukin 1<math>\beta</math></i>           | 3.7                    | 3.6                   | 31.2                     |
| <i>Interleukin 1-F8</i>                          | 8.7                    | 8.3                   | 11.4                     |
| Interleukin 6                                    | 8.3                    | 7.5                   | 12.8                     |
| Interleukin 8                                    | 3.4                    | 1.8                   | 11.7                     |
| Interleukin 12                                   | 3.5                    | 3.7                   | 4.8                      |
| Interleukin 13                                   | 5.3                    | 4.7                   | 8.7                      |
| Interleukin 17                                   | 8.1                    | 6.0                   | 8.8                      |
| Tumor Necrosis Factor Receptor 1 $\beta$         | 8.6                    | 5.5                   | 8.6                      |
| Interferon- $\omega$                             | 2.6                    | 1.7                   | 9.6                      |
| Colony stimulating factor-2                      | 5.6                    | 6.0                   | 49.6                     |
| Colony stimulating factor-3                      | 2.6                    | 1.7                   | 11.0                     |
| Platelet-derived Growth Factor- $\alpha$         | 18.1                   | 8.3                   | 14.5                     |
| Transforming Growth Factor- $\beta$ <sub>1</sub> | 3.7                    | 2.1                   | 7.3                      |
| Transforming Growth Factor- $\beta$ <sub>3</sub> | 3.3                    | 2.3                   | 3.9                      |
| Bone Morphogenetic Protein-4                     | 8.7                    | 4.2                   | <b>0.4</b>               |
| Bone Morphogenetic Protein-6                     | 1.9                    | 1.5                   | <b>0.4</b>               |
| Bone Morphogenetic Protein-8                     | 5.1                    | 2.7                   | 18.3                     |
| Growth and Differentiation Factor-8              | 4.6                    | 3.8                   | 8.5                      |
| Growth and Differentiation Factor-9              | 3.9                    | 3.5                   | 2.0                      |
| FamB3 (Pancreatic-derived Factor)                | 5.2                    | 1.9                   | 14.7                     |
| NODAL  | 3.1                    | 1.6                   | 15.4                     |

**Table 1.** Classic inflammation-associated genes identified by custom gene array as having increased or decreased expression during prostate development. Among these are IL-1 family members IL-1 $\alpha$ , IL-1 $\beta$ , and IL-1F8. IL-18 was not found to be regulated by array, while the other IL-1 family members were not included in analysis. All data represent fold change from normal 8-week dorsal-lateral prostate, and only those found statistically significant ( $p < 0.05$ , unpaired student's T-test,  $n=4$ ) are shown.

We elected to focus on the interleukin-1 (IL-1) family of cytokines because this family is critical to inflammatory and repair processes, it is known to be induced during and involved in prostate cancer and chronic prostatic inflammation, and previous anecdotal reports implicate IL-1 signaling in development-related processes in testicular biology and bone remodeling. Two of the factors showing developmentally regulated expression, IL-1 $\alpha$  and IL-1 $\beta$  are IL-1R1 ligands and are thought to play a central role in inflammatory processes. Both IL-1 $\alpha$  and IL-1 $\beta$  act through the IL-R1 receptor with similar affinities. Increased expression of

IL-1 $\alpha$  and IL-1 $\beta$ mRNA in the developing as compared to the adult prostate was confirmed by RT-PCR. **[Figure 1]**.

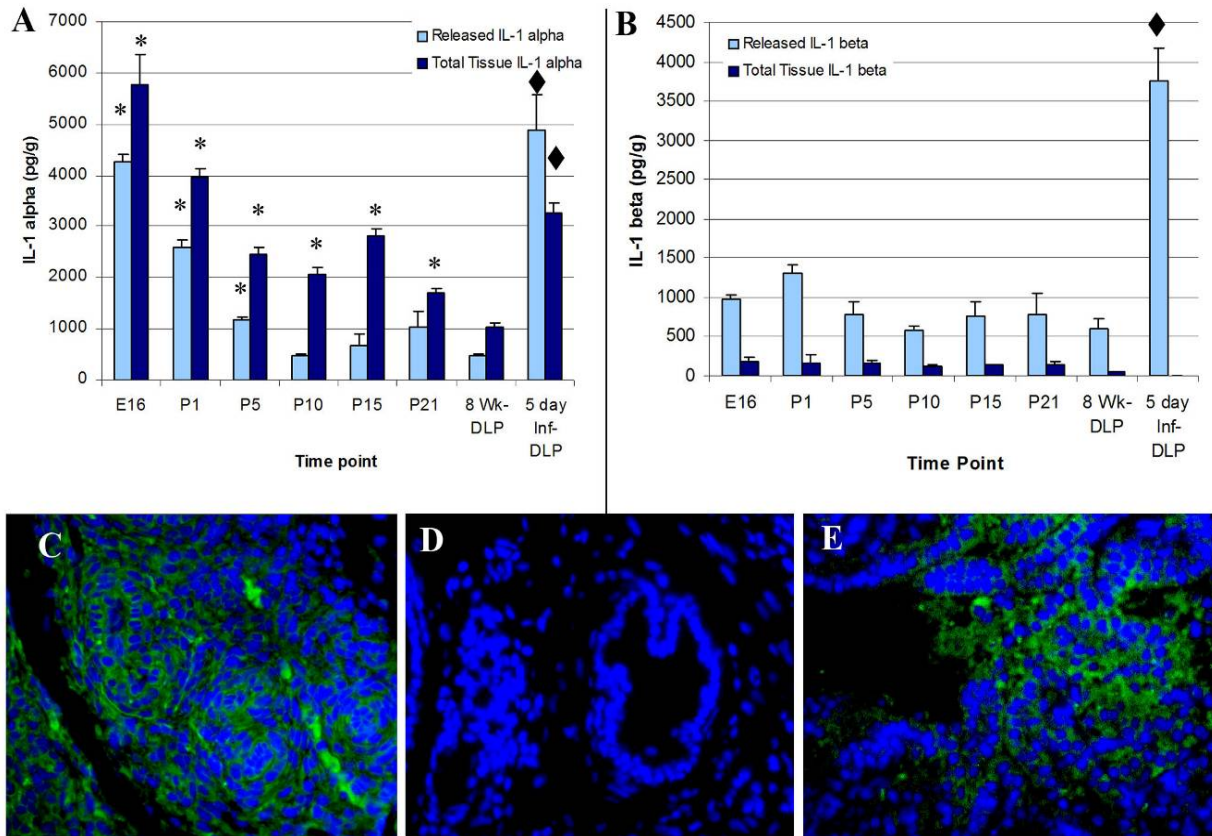
**Figure 1.** RT-PCR analysis of mRNA concentrations of IL-1 family members during prostate development. IL-1R1 ligands IL-1 $\alpha$ , IL-1 $\beta$ , IL-1F8, and IL-1F10 are all expressed at increased levels during time points of development (E16, P1, P5, P10) than during adulthood. Surprisingly, IL-1RA, the IL-1R1 antagonist, is also increased in expression during development. In contrast, IL-18 is expressed higher after reaching adulthood than during development. Statistical considerations: \*p<0.05 developmental time point, versus 8 week adult;  $\blacklozenge$ p<0.05 early developmental time-point versus P10; comparisons using unpaired student's T-test, n=6 for IL-1 $\alpha$ , IL-1 $\beta$ , IL-18, and IL-1F8; n=4 for IL-1RA and IL-1F10.



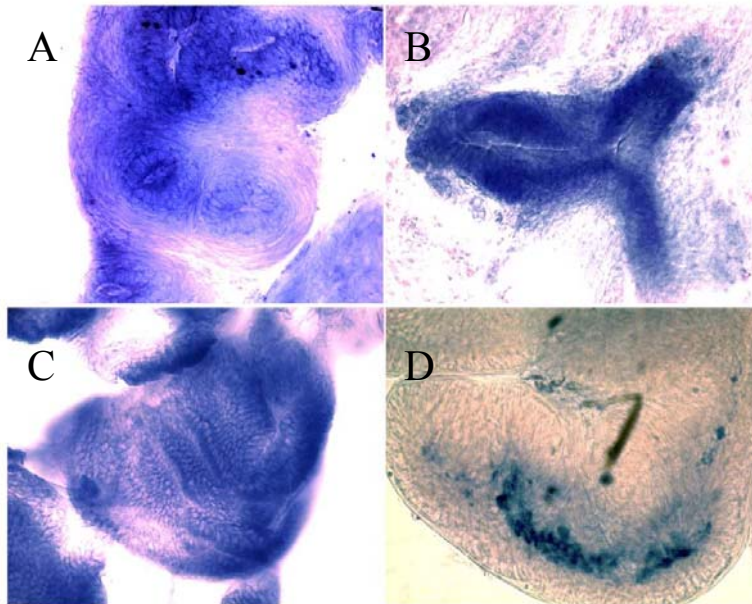
We found that IL-1 $\alpha$  total tissue concentration and released peptide are highly expressed during periods of rapid epithelial growth during prostate development. **[Figure 2A]** While developing UGS tissue releases IL-1 $\alpha$  at a rate of nearly equal to tissue total every 30 minutes at the E16 and P1, this release rate is cut in half

by P5 and to 20% by P10. In contrast, IL-1 $\beta$  concentrations remain relatively low and constant throughout prostate development, and most of the synthesized IL-1 $\beta$  is released. **[Figure 2B]** While acute inflammatory response induces IL-1 $\alpha$  concentrations and release to roughly embryonic levels, IL-1 $\beta$  synthesis and release in the inflamed prostate is significantly higher than in the developmental or normal adult prostate. These data suggest differing roles for IL-1 $\alpha$  and IL-1 $\beta$  during prostate development and hyperplastic reaction to inflammation.

**Figure 2:** IL-1 Protein concentrations and release are also regulated during development and inflammatory response. IL-1 $\alpha$  synthesis and release **[A]** is regulated during the developmental period, corresponding to time points of rapid epithelial outgrowth, and is rapidly induced during reactive hyperplasia of the adult. IL-1 $\beta$  synthesis and release **[B]** does not appear to be regulated in development, but is substantially synthesized and fully released during inflammation. Released data are pg/g per 30 minutes of release time, while total tissue concentrations are pg/g total tissue after release incubations. Immunohistochemical staining of IL-1 $\alpha$  is also evident during developmental day P5 **[C]**, absent in the adult ducts **[D]**, and reactivated in the adult **[E]**. Statistical considerations: \*p<0.05 developmental time point, versus 8 week adult;  $\blacklozenge$ p<0.05 inflamed DLP versus normal adult DLP; comparisons using unpaired student's T-test, n=4, all data points.



**B.** Hematoxylin and eosin stained sections of the P5 prostate showed no evidence of an inflammatory cell infiltrate, and immunostaining for CD-3, CD20 and F4-80 showed no evidence of T cells, B cells or macrophages, eliminating inflammatory cells as a possible source of IL-1s **[Data not shown]**. Because of this, we sought to determine the source of IL-1 ligands in the developing prostate. Localization of IL-1 mRNA by in situ hybridization showed that IL-1 $\beta$  expression is localized to the urethral urothelium during development, and little or no expression is observed in the developing prostatic ducts **[Figure 3B]**. By comparison, IL-1 $\alpha$  expression is found both in the urethral urothelium and in the developing prostatic ducts **[Figure 3A]**. In the inflamed prostate, IL- $\beta$  expression is limited to areas of inflammatory infiltrate **[Figure 3D]**, while IL-1 $\alpha$  expression is found in the hyperplastic epithelium in addition to infiltrate **[Figure 3C]**. This finding further supports differing roles for IL-1 $\alpha$  and IL-1 $\beta$ .



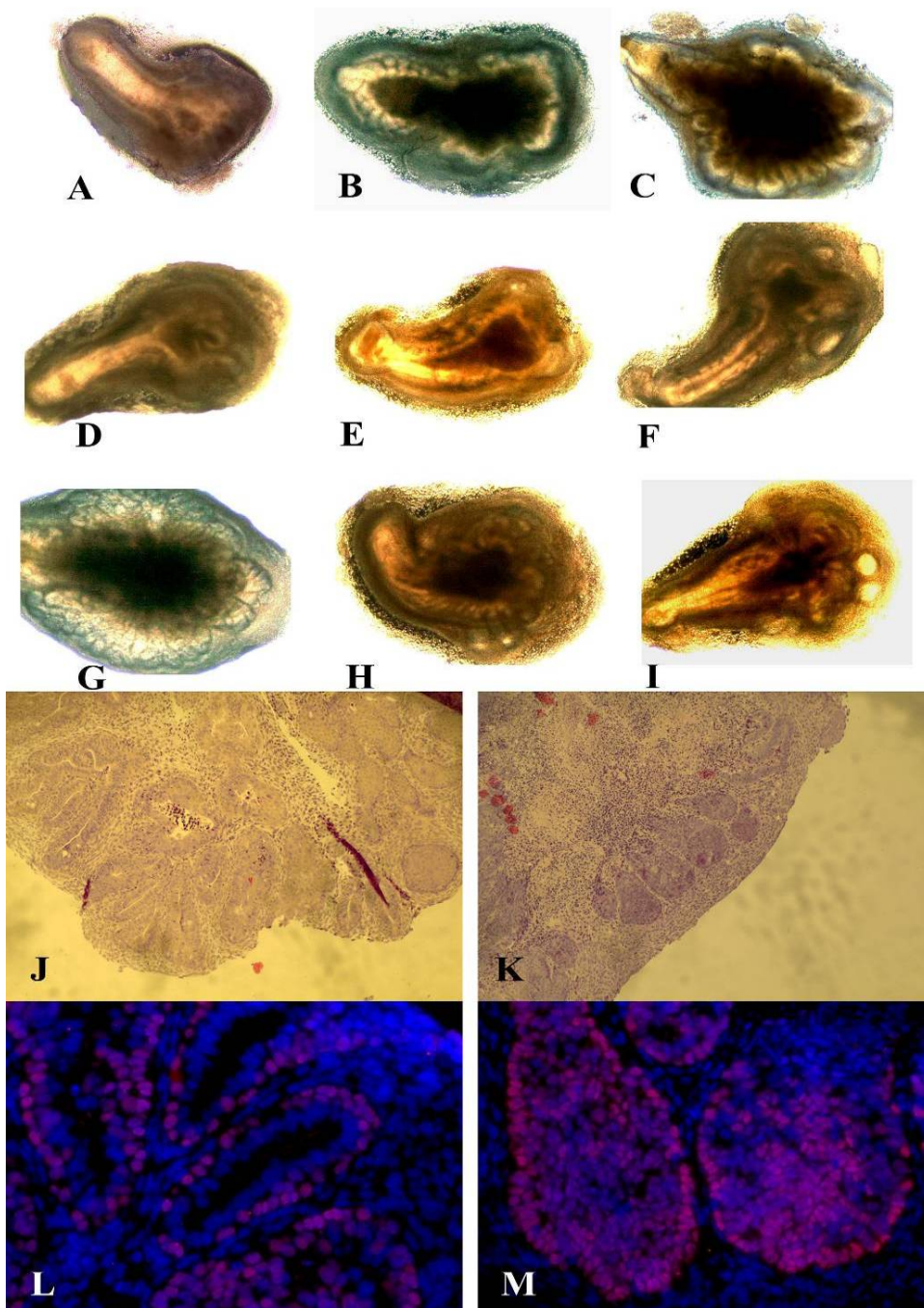
**Figure 3.** Sectional in situ hybridization for IL-1 $\alpha$  **[A,C]** shows ductal epithelial expression at developmental time point P10 **[A]** and during reactive hyperplasia of the adult **[C]**. Hyperplasia was induced by *E. coli* for 5 days. In contrast, IL-1 $\beta$  **[B,D]** expression is localized to the urethral urothelium at P10 **[B]** and inflammatory loci in the adult **[D]**. In the reactive hyperplastic response to inflammation, IL-1 $\alpha$  is found in the hyperplastic epithelium juxtaposed to areas of inflammation, while IL-1 $\beta$  and IL-18 were found in the inflammatory loci themselves, likely sourced in inflammatory cells. **[Figure 3C, D]**

**C. Fetal prostate growth is induced by IL-1 family members.** Urogenital sinuses (UGS) were removed from wild-type mice immediately prior to the time of prostatic budding [embryonic day 16, (E16)] and are grown in culture medium [see supplementary information] for up to seven days in the presence or absence of IL-1 family members, or with dihydrotestosterone (DHT, positive growth control). IL-1 $\alpha$ , IL-1 $\beta$ , IL-18 and IL-1F8 all induced growth of the cultured UGS in the absence of androgen. **[Figure 4]**. Growth induction was dose dependent and generally was apparent earlier than growth in cultures supplemented with DHT. However, IL-1 induced growth ceases earlier and overall growth is less **[Supplementary Figure 3]**. DHT induces the formation of well organized, cannulated and tapered ductal buds exhibiting a distinctive basal layer of p63+ cells. In contrast, the UGS tissues treated with IL-1 $\beta$  exhibits non-cannulated, dumb-bell shaped epithelial outgrowths characterized by an abundance of p63+ cells **[Figure 4]**. This demonstrates fundamental differences between androgen

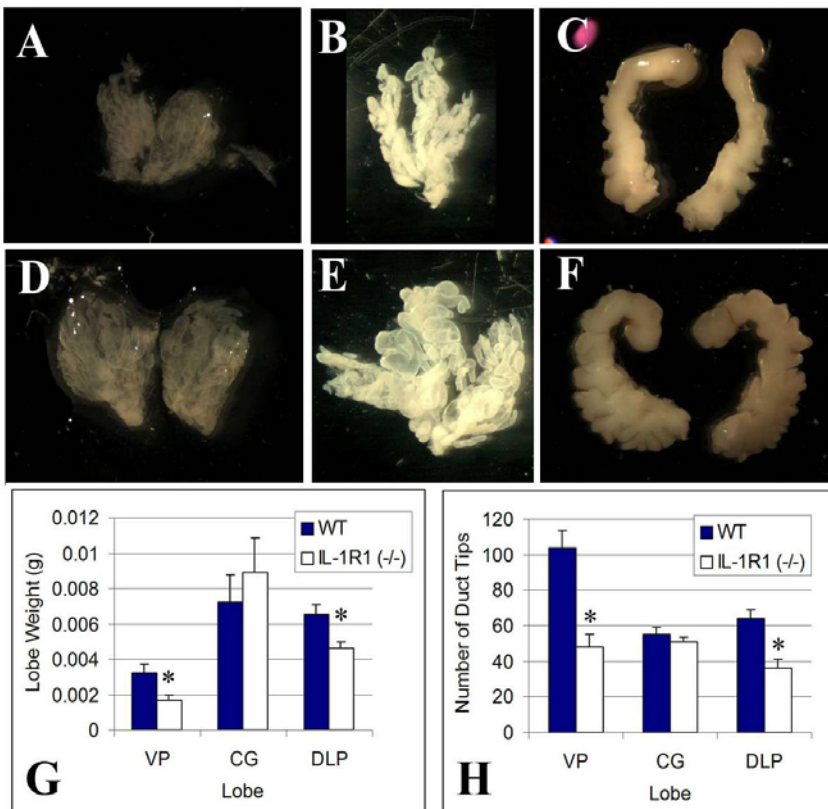


and IL-1-induced growth, and suggests that IL-1 promotes the expansion of progenitor cells, a phenomenon that has been proposed in reactive hyperplasia.

**Figure 4.** Ex vivo organ culture of E16 urogenital sinus show that IL-1 family members have the capacity to induce epithelial and stromal growth in the developing prostate. Tissues grown for 7 days in the absence of serum, hormones, growth factors, or cytokines [A] fail to outgrow. However, when supplemented with 10 nM dihydrotestosterone, [G] prostatic growth is induced. IL-1R1 ligands induce prostatic outgrowth in the absence of androgen. IL-1 $\alpha$  [B] and IL-1 $\beta$  [C] (100 ng/ml)-induced growth are shown. Simultaneous addition of the endogenous IL-1R1 antagonist IL-1RA antagonized IL-1 $\alpha$  [E] and IL-1 $\beta$  [F]-induced growth, though IL-1RA had no effect on its own [D]. IL-1F8 [H] and IL-18 [I] also induced prostatic outgrowth. Quantified 2D data of prostatic epithelial outgrowth are shown in supplementary figure 3. IL-1-induced growth [K] contrasts with DHT [J] in that the outgrowths are dumbbell-shaped rather than tapered. IL-1 [M] induces expansion of p63+ cells (magenta), while DHT [L] induces more organized expansion with p63+ cells located in the basal cell layer.

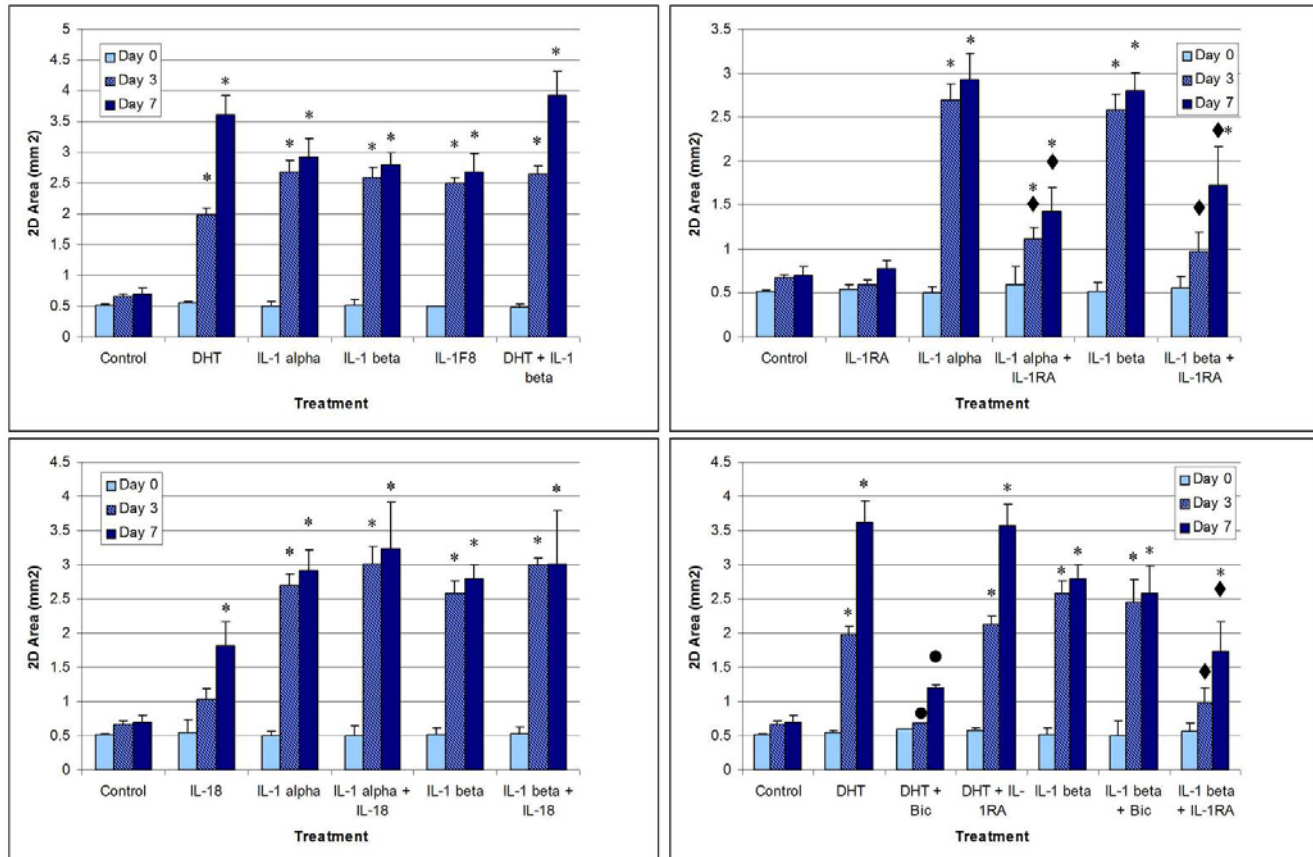


**D. IL-1 signaling is required for normal prostate development.** IL-1R1 null mice [Jackson Laboratories, MA] exhibit loss of function of the key IL-1 receptor (IL-1R1) for IL-1 $\alpha$ , IL-1 $\beta$ , and IL-1F10, and possibly IL-1F8. Adult IL-1R1 knockout mouse exhibit decreased size and branching of the ventral and dorsolateral prostate lobes [Figure 5]. There was no difference in size or branching of the coagulating gland (anterior prostate) but there was an obvious seminal vesicle phenotype characterized by decreased curvature and a reduced number of folds. The selective sparing of coagulating gland development argues against hypogonadism as a cause for the observed abnormalities of prostate and seminal vesicle development. This was confirmed by showing no difference in testosterone production from the testes of IL-1R1 (-/-) and wild-type mice at various time-points of development, as levels of testosterone per adult testes are similar to concentrations previously reported. [Data not shown] We also verified normal spermatogenesis and observed normal fertility. Further evidence against a hormonal basis for IL-1R mediated growth effects comes from comparing the responses of wild type and mutant UGS tissues in ex vivo organ culture. We first showed that IL-1-induced growth in ex vivo organ culture is not dependent upon androgen signaling, as UGS from wild-type mice treated with the selective androgen receptor antagonist bicalutamide respond similarly to IL-1 stimulation as vehicle-treated (0.1% ethanol) tissues. [Figure 6]. We then showed that IL-1R1 (-/-) mice UGS tissues respond to androgen with similar growth rates as the wild-type UGS, arguing against a deficiency in responsiveness to androgen growth stimulation. These data indicate that the effect of IL-1 on prostate growth is a primary effect that is not dependent upon androgen signaling.



**Figure 5.** IL-1R1(-/-) mice exhibited decreased growth and evidence of decreased branching morphogenesis. Ventral prostate lobes [A, D] in knockout animals [A] are half the size of wild-type animals [D] at 8 weeks of age. Further, micro-dissected ventral lobes from IL-1R1 (-/-) mice [B] exhibit half the number of branching points and duct tips as wild-type controls [E], though both strains have 3 main ducts. Quantified data for average lobe weights for all lobes is shown in panel G, while micro-dissected duct tip counts are shown in panel H. IL-1R1(-/-) mice also exhibited altered seminal vesicle development, with 8-week-old knockout animals [C] exhibiting longer, straighter vesicles with half as many folds relative to wild-type controls [F]. Statistical considerations: \*p<0.05 wild-type (WT) versus IL-1R1 (-/-), unpaired student's T-test, n=6, all groups.

**Figure 6.** Addition of IL-1 family members to ex vivo cultured urogenital sinuses induces prostatic epithelial outgrowth in the absence of androgen, as determined by quantified two-dimensional area of epithelial outgrowth. Similar growth patterns were exhibited by 100 ng/ml IL-1 $\alpha$ , IL-1 $\beta$ , and IL-1F8 in this assay [A, n=4]. Growth began more rapidly when induced by IL-1s than DHT (10 nM), and achieved near maximal size by day 3. In contrast, DHT-treated tissues continued to grow throughout the 7 day period, and reached a greater epithelial area than IL-1s. IL-1 induced prostatic growth is antagonized by concurrent addition of equimolar concentration of IL-1RA, the endogenous antagonist of the IL-1R1 receptor [B, n=4]. Despite the inverse expression relationship between IL-18 and the other IL-1s, concurrent equimolar addition of IL-18 does not antagonize IL-1-mediated growth [C, n=3]. Despite growth similarities, IL-1 signaling and androgen signaling appear to be distinct in this model, as IL-1RA does not antagonize DHT-mediated growth and 10 nM bicalutamide fails to antagonize IL-1-mediated growth [D, n=3]. Statistical considerations: \*p<0.05 versus control;  $\blacklozenge$ p<0.05 IL-1+IL-1RA versus IL-1 alone;  $\bullet$ p<0.05 DHT + bicalutamide versus DHT alone.

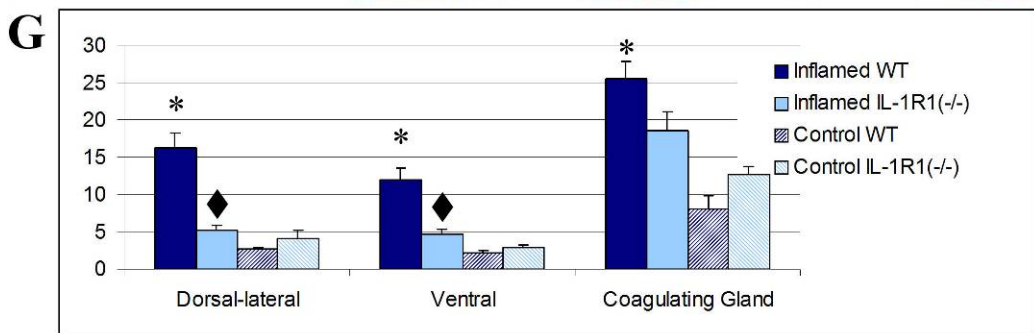
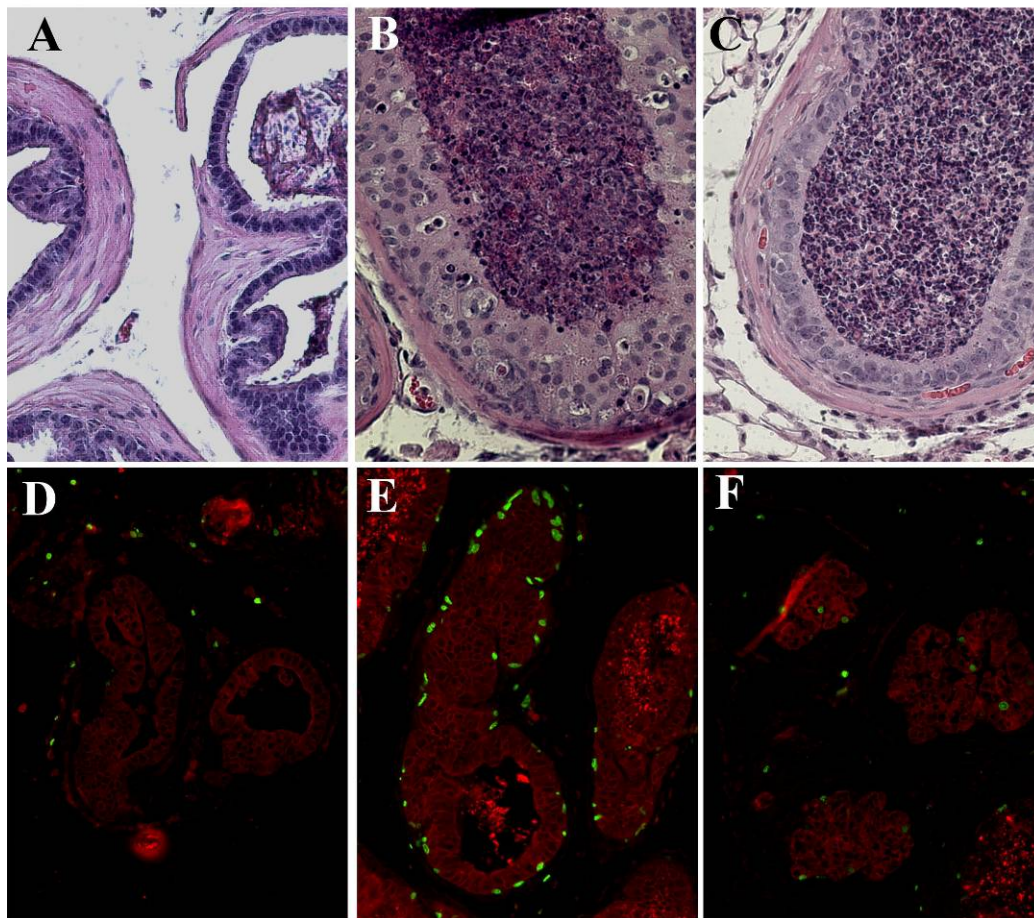


**E. Effect of IL-1R1 on acute prostatic inflammatory response.** A recently developed mouse model of bacterially-induced prostatic inflammation exhibits a reactive hyperplasia which progresses over time to severe dysplasia. To determine whether IL-1 signaling is necessary for the reactive hyperplasia associated with prostatic inflammation, we compared the response in IL-1R1 knockout mice and wild type controls. After five days of prostatic infection, wild-type animals exhibit prostatic inflammation and hyperplasia. The dorsal-lateral and ventral lobe of infected wild-type animals shows substantial hyperplastic response as evidenced by histologically evident thickened epithelium and a substantial increase in BrdU+ cells. IL-1R1 KO animals also exhibit severe acute inflammatory response as characterized by neutrophilic infiltrate, edema and epithelial slough. However, the reactive hyperplastic response in these animals is significantly reduced, as evidenced by a retaining of the psuedostratified

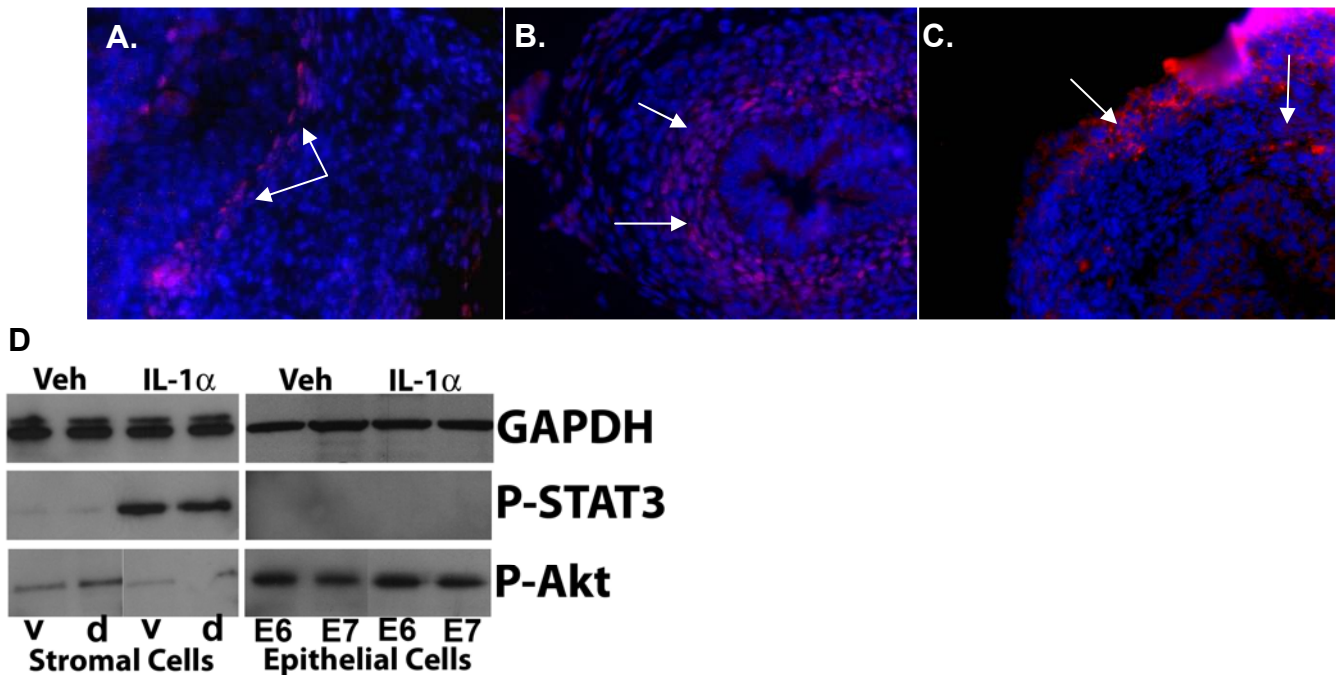


epithelium and modest increase of BrdU+ cells compared to uninfected controls [Figure 7].

**Figure 7.** IL-1R1(-/-) mice exhibit substantially reduced hyperplastic response to induced inflammation. Normal 8-week old prostate dorsal-lateral lobe architecture is shown in panel **A**, with BrdU staining (green) of normal lobes in **D**. Very little proliferation is present in the normal epithelium (control), identified by co-staining of BrdU (green) with Pan-cytokeratin (red). Wild-type mice exhibit substantial reactive hyperplasia **[B]**, and rapid epithelial rates proliferation **[E]** in response to 5 days of *E.coli*-induced inflammation. IL-1R1 (-/-) mice exhibit significantly reduced hyperplastic response to inflammation as observed by histology **[C]** and BrdU-proliferation rates **[F]**. Quantified proliferation rates are shown in panel G as the number of BrdU+ cells per duct in each lobe, and compared across inflamed and normal wild-type (WT) and IL-1R1 (-/-) mice. Statistical considerations: \*p<0.05 inflamed WT versus control WT; ♦p<0.05 inflamed IL-1R1 (-/-) versus inflamed WT, comparisons via unpaired student's T-test. Quantification of p63<sup>+</sup> cells was determined by analyzing a minimum of 8 ducts per lobe and 6 lobes per data point (n=6).

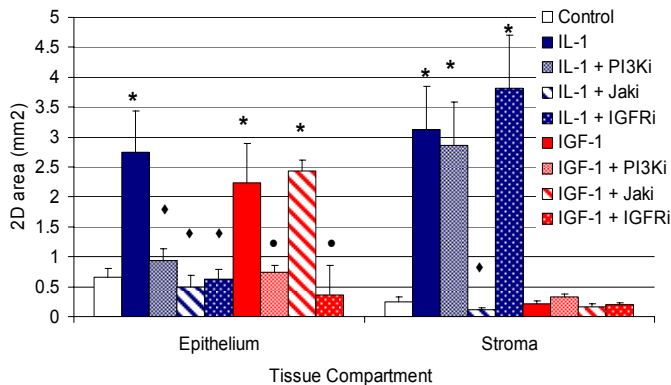
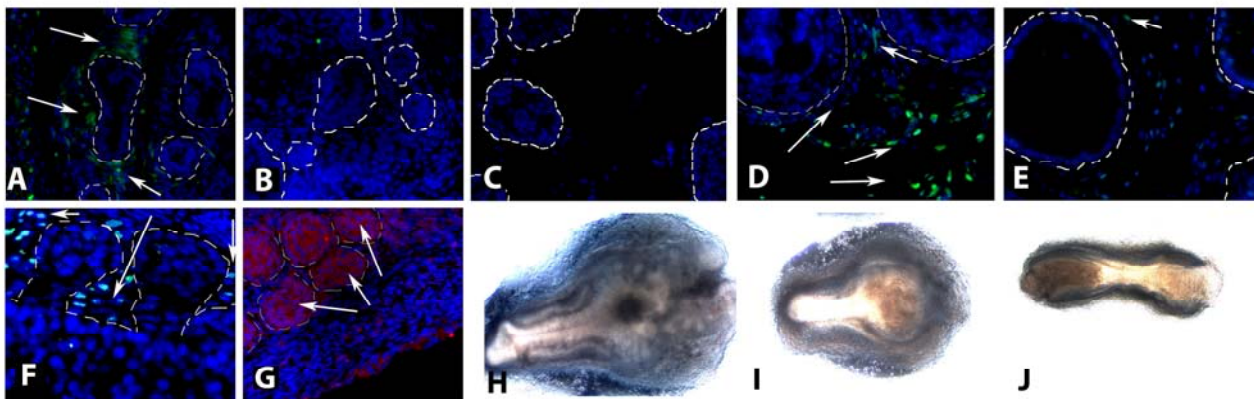


The primary receptor for IL-1 ligands, IL-1R1, is selectively activated in the stroma of the developing prostate as shown by staining for phospho-tyrosine IL-1R1 in the stroma of E16 UGS and P5 prostate. **[Figure 8 A-C]** Treatment of the cultured UGS with IL-1 $\alpha$  activated IL-1R1 in the mesenchyme but not epithelium. This activation does not occur in UGS tissues from IL-1R1 (-/-) mice (not shown). We then measured Janus-activated kinase-signal transducer and activator of transcription (Jak-STAT), phosphoinositide-3-kinase-Akt (PI<sub>3</sub>K-Akt), and nuclear factor kappa-B (NF $\kappa$ B) signaling pathways in cultured prostate stromal and epithelial cells treated with IL-1 $\alpha$ . IL-1 $\alpha$  treatment activated Jak-STAT signaling stromal cells isolated from both the ventral and dorsal-lateral prostate. **[Figure 8D]** No activation of Jak-STAT signaling occurred in epithelial cells. The PI<sub>3</sub>K-Akt pathway was active in epithelial cells in culture as indicated by phosphorylated Akt; however IL-1 treatment did not further activate this pathway **[Figure 8D]**. We did not observe activation of NF $\kappa$ B signaling in either stromal or epithelial cells, as measured by phosphorylated I $\kappa$ B or nuclear p65 localization. These data suggested a direct, stromal-specific activation of Jak-STAT signaling as a primary response to IL-1. Activation of PI<sub>3</sub> kinase signaling is abundant in epithelial cells, but does not appear to be induced by IL-1 $\alpha$  in monolayer epithelial culture.



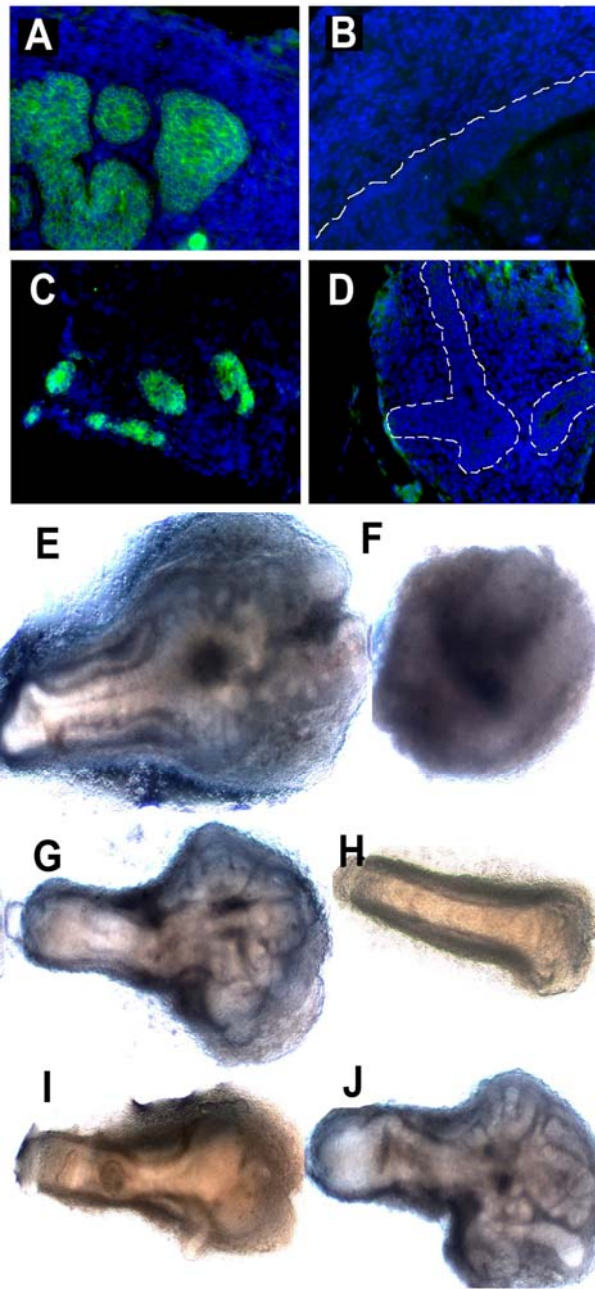
We found that Jak-STAT signaling is highly active in the stroma of wild-type 5-day-old mice, but is considerably reduced in IL-1R1 (-/-) animals. **[Figure 9 A, B]** Stromal activation of Jak-STAT signaling during inflammation is attenuated in IL-1R1(-/-) mouse prostates, **[Figure 9 C-E]** and IL-1 $\alpha$  treatment of the cultured UGS activated Jak-STAT signaling specifically in the mesenchyme. **[Figure 9F]** IL-1 treatment of the UGS also resulted in epithelial PI<sub>3</sub>K-Akt pathway activation, contrasting with the cell culture studies described above, and suggests a paracrine mechanism of activation dependent on Jak-STAT signaling. This was confirmed by treating organ cultures with IL-1 $\alpha$  in the presence or absence of Jak and PI<sub>3</sub> kinase inhibition. **[Figure 9 H-J]** Inhibition of PI<sub>3</sub>K selectively blocked epithelial expansion while Jak inhibition attenuated both epithelial budding and stromal expansion. The selective activation of IL-1R1 in the prostatic stroma, the cell and organ culture studies suggesting direct activation of stromal Jak-STAT and indirect activation of epithelial PI<sub>3</sub>K-Akt pathway activation, and the dichotomous effect of the Jak and PI<sub>3</sub>K inhibitors are best explained by a paracrine signaling loop in which IL-1 acts upon the stroma in a Jak-dependent fashion to produce epithelial PI<sub>3</sub>K-Akt pathway activation.

**Figure 9**



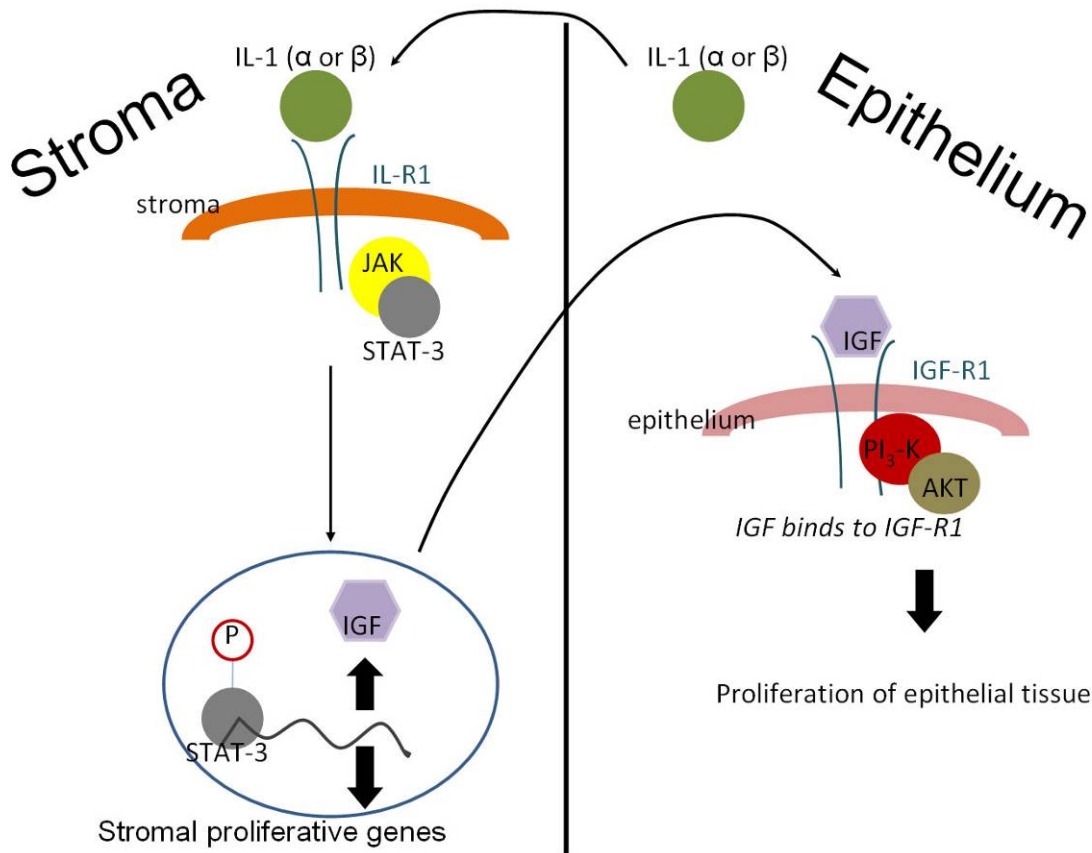
**Figure 10.** Quantitative analysis of epithelial or stromal outgrowth induced by 10 ng/ml IL-1 $\alpha$  or IGF-1 in ex vivo cultured urogenital sinus tissues in the absence of androgen. Growth was quantified by two-dimensional area of epithelial outgrowth in photomicrographs taken of cultured tissues on Day 3. [n=4]. IL-1 induced epithelial outgrowth was inhibited by both PI<sub>3</sub>K inhibition (PI<sub>3</sub>Ki, LY294002 [3  $\mu$ M]) and IGF1R1 antagonism (IGF1Ri, PPP [30 nM]) while stromal outgrowth was inhibited by Jak inhibition (Jaki, Pyridone-6, [30  $\mu$ M]). IGF-1 only induced outgrowth of the epithelium, and this was antagonized by PI<sub>3</sub>K inhibition but not Jak inhibition. Data presented are mean  $\pm$  s.e.m. Statistical considerations (Anova): \*p<0.05 versus control; ◆p<0.05 IL-1+ inhibitor versus IL-1 alone; ●p<0.05 IGF-1+inhibitor versus IGF-1 alone.





IL-1 treatment of UGS in organ cultures substantially induced activation of IGF1R in the UGS epithelium as determined by phosphotyrosine-IGF1R. **[Figure 11A]** This finding suggests activation of paracrine IGF signaling by IL-1 and, consistent with this inference, we found that concurrent treatment with Jak inhibitors abrogates epithelial IGF1R activation. **[Figure 11B]** Developing prostates (P5) in vivo expressed epithelial-specific IGF1R activation that is significantly attenuated in the epithelium of IL-1R1 knockout mice. **[Figure 11 C, D]** In cultured E16 UGS tissues treated with IL-1, antagonists to the primary IGF-1 receptor (IGF1R) demonstrated a selective attenuation of IL-1-induced epithelial growth. **[Figure 11 E, F; quantified data in figure 10]** This is consistent with the finding that IGF-1 itself selectively induces prostate epithelial growth in organ culture. **[Figure 11G]** Using pathway inhibitors, we found that IGF-1 induced epithelial growth is PI<sub>3</sub>K dependent but not Jak-STAT dependent, echoing the selective effect of PI<sub>3</sub>K inhibition on IL-1 induced epithelial growth, **[Figure 11 I, J; figure 10]**.

**Figure 12.** Schematic representation of the findings of the present study. IL-1 family members expressed in the epithelium during prostate development or either epithelium or leukocytes during inflammation signals to the stroma where IL-1R1 is expressed. Activation of IL-1R1 causes Jak-STAT pathway activation resulting in the expression of genes, including (but not limited to) IGF. IGF signals to the epithelium in both developing and hyperplastic prostates, resulting in PI<sub>3</sub>K-Akt pathway activation, epithelial proliferation, and tissue expansion.





## Research Accomplishments

The following research accomplishments were attained with this proposal *in the first year of work*:

1. We determined that inflammatory mediators induce proliferation of prostate epithelial cells and prostate cancer cells in culture, in a PI<sub>3</sub>K cascade dependent fashion. [Task 1, parts A and B]
2. We found that acute inflammatory signals induce the PI3K cascade in the epithelium of the prostate in ex vivo organ culture; Jak-STAT is induced in the stroma, and this stromal induction of Jak-STAT is necessary for the epithelial induction of PI3K and epithelial growth. [Task 2, part A]
3. We found that inflammatory mediators induce epithelial proliferation and hyperplasia in tissues. [Task 2, part B]
4. We determined that Akt is activated (phosphorylated) and PTEN is reduced in expression during chronic prostatic inflammation, indicating induction of the PI3K cascade during inflammation in vivo. [Task 3, part A]
5. We found that chronic prostatic inflammation induces the PI<sub>3</sub>K pathway in the epithelium and the Jak-STAT pathway in the stroma in mouse prostates.
6. We found that epithelial proliferation and hyperplasia associated with inflammatory tissue damage is dependent upon IGF-1-induced PI3K pathway induction.
7. We discovered that a myriad of inflammatory mediators are highly expressed during normal development of the prostate, are diminished in expression in the adult, and are highly induced during acute and chronic prostatic inflammation.
8. We defined the IL-1 family of inflammatory cytokines as a critical family of so-called inflammatory mediators that is highly expressed during prostate development.
9. We determined that IL-1 family members can drive the growth of prostatic ducts in an organ culture model of prostatic outgrowth, in the absence of androgen.
10. We determined that putative prostatic epithelial cells are expanded by IL-1 in vitro.
11. We characterized a key prostatic growth defect in mice deficient for IL-1R1 receptor, the key receptor for IL-1 family members.

12. We discovered that reactive hyperplasia secondary to prostatic inflammation induction is significantly reduced in animals deficient in IL-1R1.

13. We discovered a novel epithelial-stromal signaling interaction involving interleukin-1 signaling during prostate growth. IL-1 is produced in the epithelium or leukocytes and signals to the stroma to induce IGF-1 expression in a STAT-3 dependent fashion. IGF-1 signals back to the epithelium to induce proliferation in a PI3K-Akt dependent fashion.

## REPORTABLE OUTCOMES

### Manuscripts:

**Jerde TJ**, Bushman W: Interleukin-1 induced epithelial proliferation in prostate development and hyperplasia is mediated by stromal insulin-like growth factor-1 induction. ***Science Signaling***. **In press**, May 2009

**Jerde TJ**, Wong L, Wu Z, Theodorescu D, Bushman W: Bone morphogenetic signaling sustains Phosphatase and Tension analogue (PTEN) protein expression in prostate epithelial cell lines. ***Cancer Research***, **Submitted**, 2009.

Boehm BJ, **Jerde TJ**, Sullivan R, Bushman W. Characterization of the reactive hyperplastic response to acute inflammation in the mouse prostate. ***Journal of Urology***, **Submitted**, June 2009.

**Jerde TJ**, McFarland EK, Bushman W. Insulin-like growth factor-1 signaling drives the acute reactive hyperplastic response to inflammation in the mouse prostate. ***Nature Medicine***, **Submission planned**, June 2009.

**Jerde TJ**, McFarland EK, Bushman W. Inflammatory hyperplasia in the mouse prostate is potentiated by stromal and leukocytic hedgehog signaling. ***Journal of Pharmacology and Experimental Therapeutics***, **Submission planned**, July 2009.

Elkhwaji JE, Zhong W, Hopkins WJ, Bushman W. Chronic bacterial infection and inflammation incite reactive hyperplasia in a mouse model of chronic prostatitis. ***Prostate* 67(1):14-21**, 2007

### Abstracts and Presentations of the funded work from this proposal:

**Jerde TJ**, Landry MM, Bushman W. Decreased prostatic branching in interleukin receptor knockout mice reveals a key role for inflammatory mediators in prostate development. Presented at the Keystone Symposium on "*Signaling Pathways in Cancer and Development*" March 24, 2008-March 29, 2008, Steamboat Springs, CO.

**Jerde TJ**, Landry MM, Bushman W. Interleukin-1 signaling plays a critical role in prostate development. Presented at the Annual meeting of the American Urological Association, Orlando, FL, May 20-25, 2008.

**Jerde TJ**, McFarland EK, Bushman W. Interleukin-1 induced epithelial proliferation in prostate development and hyperplasia is mediated by stromal insulin-like growth factor-1 induction. Presented at the Society of Basic Urological Research (SBUR) 2008 Annual Fall Meeting, Phoenix, AZ, November 20-23, 2008.

**Jerde TJ, McFarland EK, Bushman W.** Interleukin-1 induced epithelial proliferation in prostate development and hyperplasia is mediated by insulin-like growth factor-1 induction. Presented at the Annual meeting of the American Urological Association, April 27<sup>th</sup>, 2009, Chicago, IL, USA.

**Awards obtained from the funded work:**

**Keystone Symposium on Signaling Pathways in Cancer and Development, 2008-Travel Award-Podium Selection.** “Decreased prostatic branching in interleukin receptor knockout mice reveals a key role for inflammatory mediators in prostate development.” March 24- 29, 2008, Steamboat Springs, CO, USA.

**Society of Basic Urological Research (SBUR) 2008 Annual Fall Meeting-Travel Award-Best Poster Submission.** “Interleukin-1 induced epithelial proliferation in prostate development and hyperplasia is mediated by stromal insulin-like growth factor-1 induction.” November 20-23, 2008, Phoenix, AZ, USA.

**Best Poster of Session: Prostate Cancer Basic Research. The Annual Meeting of the American Urological Association** “Interleukin-1 induced epithelial proliferation in prostate development and hyperplasia is mediated by insulin-like growth factor-1 induction.” April 27<sup>th</sup>, 2009, Chicago, IL, USA.

**Animal Model**

We have developed a mouse model of acute prostatic inflammation that produces a reproducible reactive hyperplasia excellent for studying the molecular mechanisms behind reactive epithelial growth. This has already been used in genetically modified mice (IL-1R1 [-/-]) and is optimal for use in other genetic models.

## CONCLUSION

Our data show that PI3K signaling is involved in rapid epithelial proliferative states of the prostate, including the hyperplastic response during inflammation known as “reactive hyperplasia”. Prostatic epithelial cells are induced to proliferate by IL-1 in a PI3K-dependent fashion. Further, inflammation induces reactive hyperplasia, and the PI3K cascade is induced in this condition relative to control tissue. Further studies are in progress to determine the dependence of reactive hyperplasia on PI3K signaling. Interestingly, prostate stromal cells are associated with induction of Jak-STAT signaling in response to inflammatory signals rather than PI3K. Future studies will be expanded to study the interrelationships between the epithelium and stroma in inflammation.

Our expanded data show that that multiple cytokines generally considered to be inflammatory mediators are expressed in the developing prostate. While there have been anecdotal reports of specific interleukins being expressed in one developing tissue or another, evidence for a wholesale expression of inflammatory mediators during development of a non-immune soft tissue organ such as the prostate has not been previously presented. The functional importance of this expression is shown by the clear cut-growth inducing effects of the IL-1 ligands in culture and the hypomorphic phenotype of the IL-1R null mouse prostate. These observations demonstrate that cytokines heretofore considered to have a primary role in inflammation may have equally important roles in development. It has been suggested that re-activation of developmental signaling pathways such as Hedgehog and Wnt signaling occurs in the regenerative response to injury and plays an important role in progenitor cell proliferation. Similarly, our studies suggest that cytokines such as IL-1 ligands, which exert growth effects in normal development, may exercise a similar activity in the inflammatory response to injury when expressed either from the tissue itself. The capacity for cytokines which are expressed in conditions of chronic inflammation to directly stimulate the proliferation of undifferentiated p63+ prostate epithelial cells is a provocative finding that not only helps explain the progressive hyperplasia we observe in chronic prostatic inflammation but also suggests a mechanism by which inflammation may expand the pool of progenitor cells which are the target for malignant transformation. These findings, taken in the context of abundant evidence linking inflammatory signaling to promotion of tumor growth, lead us to postulate that the hyperplasia associated with chronic inflammation and the trophic effect of inflammatory mediators on tumor growth reflects a fundamental symmetry in the biological processes of development, chronic inflammation and cancer and the conserved role of inflammatory mediators in promoting epithelial cell proliferation.