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Induction of proinflammatory response in prostate cancer epithelial cells by activated macrophages

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Abstract

Emerging evidence indicates that chronic inflammation plays an important role in prostate carcinogenesis. Yet to date the precise molecular and cellular mechanisms linking inflammation to carcinogenesis remains unclear. The purpose of this study was to determine the local contribution of prostate epithelial cells to the inflammatory process. We characterized the inflammatory response elicited directly by prostate epithelial cells using an in vitro culture system in which androgen-dependent LNCaP prostate cancer epithelial cells were exposed to conditioned media from LPS-activated THP-1 macrophages. Upon exposure to activated macrophage conditioned media, LNCaP cells elicited a local proinflammatory response, as evidenced by NFκB activation, and the production of proinflammatory cytokines TNFα, IL-1β, and IL-6. Furthermore, we observed a significant upregulation of the adhesion molecule VCAM-1 and nuclear estrogen receptor α (ERα), two biomarkers that correlate with tumor immune evasion and tumor progression. Our results suggest that prostate epithelial cells may play a significant role in sustaining and amplifying the inflammation process through NFκB activation and local production of proinflammatory cytokines that results in the recruitment and activation of additional immune cells in the prostate. At the same time, increased expression of VCAM-1 and ERα in prostate epithelial cells upon exposure to inflammatory conditions highlights the potential link between chronic inflammation and its involvement in promoting prostate cancer carcinogenesis.

Keywords

Prostate cancer; inflammation; immune response; macrophage

1. Introduction

Prostate cancer is the most commonly diagnosed non-cutaneous cancer and the second leading cancer-related cause of death for men, responsible for nearly 30,000 deaths per year in the United States [1]. In 2007, it is estimated that over 200,000 new cases of prostate cancer are diagnosed. Despite its high prevalence, the precise mechanisms and etiological factors leading to prostate cancer progression remain unclear. More recently, chronic inflammation has gained

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attention as a potential important component in the progression of cancer. It is estimated that approximately 20% of all human cancers are caused by chronic infection and inflammation, resulting from environmental insults such as infectious agents, or exposure to dietary carcinogens [2]. Chronic inflammation is strongly implicated in the development of several cancers, including colon, liver, bladder, and gastric cancer [3]. Emerging evidence indicates that chronic inflammation may also play an important role in prostate carcinogenesis. A positive association between clinical prostatitis (inflammation of the prostate) and prostate cancer risk has been reported [4;5]. Other studies indicate a reduction in the risk of prostate cancer with long-term use of anti-inflammatory agents such as aspirin and non steroidal anti-inflammatory drugs [6;7]. Histological analysis reveals that prostatic lesions are often associated with increased presence of inflammatory cells, termed proliferative inflammatory atrophy (PIA) [8;9]. These PIA lesions are commonly found in the peripheral zone of the prostates in ageing men, its location coincides with where prostate cancer predominantly develops. In addition, transitions between areas of PIA to high grade prostatic intraepithelial neoplasia (PIN) lesions and adenocarcinoma have been observed, indicating active inflammation is present at sites where prostate cancer develops. Taken together, these studies underscore the involvement of chronic inflammation in promoting prostate cancer development.

The initial trigger for prostatic inflammation remains unclear. Candidate sources include infection by pathogens, cell injury, dietary factors, and hormonal imbalance, all of which can potentially activate the innate immune system and trigger a proinflammatory response, and subsequently recruit components of the adaptive immune system that can amplify inflammatory responses [2]. Multiple immune cell populations are involved in the inflammatory response. Among them, macrophages play a key role in chronic inflammation. Indeed, tumor-associated macrophages are a major component of immune infiltrates present in the tumor microenvironment [10;11]. Macrophages produce a host of cytokines and growth factors upon stimulation and activation, such as during encounter with bacteria pathogens, and recruit additional immune cells to the inflammation site which amplify the inflammatory response. Both epidemiological and histological studies implicate the role of chronic inflammation in prostate cancer development. While the molecular and cellular mechanisms of how this is accomplished have yet to be clearly defined, dysregulation of the transcription factor NF κ B has been proposed to be one putative molecular mechanism leading to chronic inflammation and cancer [12]. NF κ B regulates the expression of numerous genes during the inflammatory process in immune cells [13]. At the same time, constitutive or increased activation of NF κ B has been observed in prostate cancer cells [14;15]. This potentially can lead to the amplification of the inflammatory response by providing a positive feedback signal to immune cells present in the tumor environment.

While increasing evidence implicates the role of chronic inflammation in prostate cancer carcinogenesis, it remains unclear how the prostate epithelial cells are directly involved in amplifying the inflammatory process which results in chronic inflammation. Dissecting the relative roles contributed by activated immune cells such as macrophages and prostate epithelial cells during chronic inflammation will aid in our understanding of how the proinflammatory response is initiated and amplified during prostate carcinogenesis. The goal of this study is to examine the effects of activated macrophages on NF κ B activation, production of proinflammatory cytokines, adhesion molecule and hormone receptor expression in the prostate cancer epithelial cells LNCaP. This data will provide evidence for the involvement of prostate epithelial cells in initiating and sustaining chronic inflammation that leads to the development and progression of prostate cancer.

2. Materials and Methods

2.1. Cell culture

Human monocytic cell line THP-1 and androgen-dependent prostate cancer epithelial cells (LNCaP) were obtained from American Type Tissue Collection (Manassas, VA). Cells were grown and maintained in RPMI 1640 with 10% Fetal Bovine Serum (FBS). Both cell lines were maintained in humidified incubators with 5% CO₂ at 37 °C.

2.2 Treatments

THP-1 monocytes were seeded in T75 tissue culture flasks at 3×10^6 cells per flask, and differentiated into macrophages in RPMI media containing 5 ng/ml phorbol 12-myristate 13-acetate (PMA) for 48h according to previously established protocols [16]. After differentiation, THP-1 macrophages were either left untreated, or activated with low dose lipopolysaccharide (LPS) at 10 ng/ml. Both LPS and PMA were obtained from Sigma (St. Louis, MO). For real time PCR analysis, THP-1 cells were harvested after 3h LPS treatment. For collection of conditioned media, culture supernatants from LPS-activated THP-1 macrophages were collected after 48 h incubation, filter sterilized, and stored at -20 °C until ready for use. Conditioned media were analyzed for proinflammatory cytokines production by ELISA, and subsequently used to treat LNCaP cells.

LNCaP cells were seeded at 1×10^6 cells in 10 cm tissue culture dishes one day prior to treatment. Cells were 1) left untreated, or treated with 2) 10 ng/ml LPS, 3) conditioned media from untreated THP-1 macrophages, or 4) conditioned media from LPS-activated THP-1 macrophages. All treatments were done in triplicates. For NFκB and estrogen receptor α (ERα) analysis, cells were treated for 3 h and harvested for Western analysis. For proinflammatory cytokines and VCAM-1 analysis, cells were treated for 24 h and harvested for ELISA, and Western or flow cytometry analysis, respectively.

2.3. Western analysis

LNCaP protein extracts (nuclear and cytosolic protein fractions) were isolated using Nuclear Extract Kit from Activ Motif (Carlsbad, CA). Protein concentrations were determined using DC Protein Assay (Bio-Rad, Hercules, CA). Twenty-five μg protein were separated by SDS-PAGE under standard conditions and were transferred to nitrocellulose membrane for Western analysis. Antibodies used for detection include rabbit anti-NFκB p65, rabbit anti-IκBα, rabbit anti-VCAM-1, mouse anti-ERα (all from Santa Cruz Biotechnology, San Diego, CA), and mouse anti-β-actin (Sigma). Bound antibodies were detected using either goat anti-mouse IgG-HRP or goat anti-rabbit IgG-HRP (San Cruz Biotechnology), and developed with SuperSignal West Femto chemiluminescent substrate (Pierce, Rockford, IL). Images were acquired on Alpha Innotech photodocumentation system (Alpha Innotech, Hayward, CA) and analyzed using Image J software (NIH, Bethesda, MD).

2.4. RNA isolation, cDNA synthesis, and real time quantitative PCR

Total RNA from THP-1 cells were isolated using Trizol reagent (Invitrogen). One microgram total RNA was reverse transcribed into cDNA using SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen). Real time PCR was performed using primers specific for mouse TNFα (forward: 5'- CTGTAGCCCACGTCGTAGCA-3', reverse: 5'- GTGTGGGTGAGGAGCACGTA-3'), IL-1β (forward: 5'- AAGATGAAGGGCTGCTTCCAA-3', reverse: 5'- TGAAGGAAAAGAAGGTGCTCATG-3'), IL-6 (forward: 5'- GAGGATAACCACTCCCAACAGACC-3', reverse : 5'- AAGTGCATCATCGTTGTTTCATACA-3'), iNOS (forward: 5'-

TGCCCCCTTCAATGGTTGGTA-3', reverse: 5'-GAAACTATGGAGCACAGCCACAT-3'), and 18S ribosomal RNA (18S) (forward: 5'-CCGCAGCTAGGAATAATGGAAT-3', reverse: 5'-CGAACCTCCGACTTTCGTTCT-3'). Real time PCR reactions were performed using DyNAmo HS SYBR Green qPCR kit (New England Biolabs, Ipswich, MA). Gene copies were determined using the standard curve method. A standard curve was generated from serial dilutions of purified plasmid DNA that encoded for each gene of interest. Data represent averaged fold-change of LPS-treated THP-1 cells compared to untreated THP-1 cells after the copy number of the gene of interest was normalized to the copy number of 18S housekeeping gene.

2.5. Cytokine measurements

THP-1 culture supernatants or LNCaP cytosolic protein extracts were collected for cytokine analysis by ELISA. The human cytokines IL-1 β , IL-6, and TNF α were detected using cytokine-specific ELISA Ready-SET-Go! reagents from eBioscience.

2.6. Assessment of oxidant production

Reactive oxygen species (ROS) was measured using 2',7'-dichlorodihydrofluorescein diacetate (DCFH, Sigma), a dye that is cleaved and trapped intracellularly where it is subsequently oxidatively modified by intracellular H₂O₂ and peroxidase to produce fluorescent 2',7'-dichlorofluorescein (DCF). Untreated or LPS-treated THP-1 macrophages were incubated with 20 μ M DCFH for 15 mins at 37. Cells were washed in PBS and harvested for DCF analysis by flow cytometry. In addition, nitrite formation was used as an indirect measure of nitric oxide (NO) production. Nitrite concentration in culture supernatant was determined by using standard Griess assay, and quantitated using known nitrite standards as described in [17].

2.7 Flow cytometry analysis

THP-1 macrophages and LNCaP cells were harvested in 0.25% trypsin solution (Invitrogen) and resuspended in single cell suspension in flow cytometry buffer (PBS, 2% FBS, 1mM EDTA). For DCFH-labeled THP-1 macrophages, cells were analyzed directly after cell harvest for the relative fluorescent intensity of DCF. For LNCaP cells, cells were first incubated with PE-anti human VCAM-1 for 30 mins on ice in the dark (eBioscience, San Diego, CA). After extensive washing, cells were resuspended in buffer for flow cytometry analysis. Data was acquired using FACSCalibur (BD Biosciences, San Jose, CA). Data analysis was performed using Summit software (DakoCytomation, Fort Collins, CO).

2.8. Statistical analyses

Statistical differences in means between treatment groups were compared using one-way ANOVA with Tukey's post test, or unpaired Student's t test using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA).

3. Results

3.1. Low dose LPS induces the production of proinflammatory cytokines in THP-1 macrophages

We are interested in characterizing the effects of proinflammatory soluble mediators produced by activated macrophages on prostate epithelial cells. THP-1 monocytes were differentiated into macrophages with PMA treatment, and subsequently stimulated by LPS to become activated macrophages. THP-1 macrophages were activated with low dose LPS (10 ng/ml) in order to resemble physiological concentrations of LPS exposed, for example, during bacterial infection [16;18]. LPS-treated THP-1 macrophages rapidly upregulates the mRNA expression of the prototypical proinflammatory cytokines (IL-1 β , IL-6, and TNF α) within 3h of LPS

treatment (Table 1). No significant expression of nitric oxide production was detected, as determined by the mRNA expression of inducible nitric oxide synthase (iNOS). The production of proinflammatory cytokines in THP-1 macrophages was confirmed by quantifying the level of protein expression in LPS-treated THP-1 culture supernatant. Upon low dose LPS treatments, significant amounts of IL-1 β (18.6 $\pm$ 1.1 pg/ml), IL-6 (17.1 $\pm$ 1.2 pg/ml), and TNF α (1008 $\pm$ 12.9 pg/ml) were detected in culture supernatants of LPS-treated THP-1 macrophages 48 h after LPS treatment (Figure 1). The production of ROS and NO were assessed by DCF staining and Griess assay, respectively. In agreement with real time PCR results, no significant production of NO was detected in THP-1 macrophages treated with low dose LPS (data not shown). In addition, no ROS was detected as evaluated by DCF staining (data not shown). The lack of ROS and NO production in THP-1 macrophages was likely due to the use of low dose LPS that was not sufficient to induce oxidant production. Unstimulated THP-1 macrophages did not produce any detectable levels of cytokines. Culture supernatants from untreated and LPS-treated THP-1 macrophages were collected, and were used in subsequent experiments as “THP-1 conditioned media”.

3.2. Activation of nuclear NF κ B p65 and degradation of cytosolic I κ B α in LNCaP cells treated with LPS-activated THP-1 macrophage conditioned media

NF κ B is a family of transcription factors that regulates the expression of multiple genes involved in inflammation, and dysregulation of NF κ B is implicated in cancer development [14;15]. NF κ B activation results in the phosphorylation and degradation of the inhibitory molecule I κ B (α and β), mediated by I κ B kinase. Degradation of I κ B leads to the nuclear translocation and subsequent nuclear binding of NF κ B complex including p65 and p50 to consensus sequences and the induction of gene transcription, including the upregulation of proinflammatory cytokines and adhesion molecules. We sought to determine the effects of proinflammatory cytokines secreted by LPS-activated THP-1 macrophages on the NF κ B status of LNCaP cells, an androgen-dependent prostate epithelial cancer cell. Specifically, NF κ B activation was determined by quantifying the relative amounts of cytosolic I κ B α and nuclear NF κ B p65 by Western analyses following stimulations. LNCaP cells were treated with conditioned media from low dose LPS-stimulated or unstimulated THP-1 macrophages. NF κ B activation was determined 3 h after treatment. Direct effects of low dose LPS on LNCaP cells was determined by treating additional LNCaP cells with 10 ng/ml LPS. Baseline NF κ B activation was determined in untreated LNCaP cells. A significant increase in nuclear NF κ B p65 protein expression was observed in LNCaP cells treated with conditioned media from THP-1 macrophages stimulated with LPS as compared to LNCaP cells treated with conditioned media from unstimulated THP-1 macrophages and untreated controls ($p < 0.01$) (Figure 2). A similar increase in nuclear NF κ B p50 protein expression was also observed (data not shown). Nuclear translocation of NF κ B p50 and p65 correlated with a concomitant degradation of the inhibitory protein I κ B α in the cytosol ($p < 0.01$). NF κ B activation in LNCaP cells was not due to residual LPS present in the THP-1 macrophage conditioned media, as direct treatment of LNCaP cells with LPS did not result in detectable changes in NF κ B proteins.

3.3. Production of proinflammatory cytokines in LNCaP cells treated with LPS-activated THP-1 cells conditioned media

One of the consequences of NF κ B activation is the induction of proinflammatory cytokines expression in immune cell populations. However, it is unclear whether prostate epithelial cells themselves can similarly produce proinflammatory cytokines upon NF κ B activation. We assessed the production of IL-1 β , IL-6, and TNF α in LNCaP cells treated with LPS-activated THP-1 macrophage conditioned media after 24 h by ELISA. Significant production of IL-1 β (30.7 $\pm$ 5.7 pg/ml), IL-6 (17.1 $\pm$ 0.3 pg/ml), and TNF α (13.6 $\pm$ 3.0 pg/ml) were observed in LNCaP cells treated with LPS-activated THP-1 macrophage conditioned media ($p < 0.01$) (Figure 3). The increase in proinflammatory cytokines was not due to direct effects of LPS, as

there was no significant increase in cytokine production in LPS treated LNCaP cells compared to untreated control. Furthermore, the increase in proinflammatory cytokines was not due to nonspecific uptake of IL-1 β , IL-6, and TNF α from the conditioned media into LNCaP cells. Quantitative real time PCR results indicated that all three cytokine genes being tested were significantly induced compared to controls (data not shown).

3.4. Upregulation of adhesion molecule VCAM-1 in LNCaP cells treated with LPS-activated THP-1 cells conditioned media

VCAM-1 is an adhesion molecule that plays a direct role in tumor progression by promoting tumor escape from anti-tumor immunity [19]. Furthermore, increased expression of the adhesion molecule VCAM-1 is a biomarker that predicts prostate cancer relapse [20]. Since VCAM-1 expression in endothelial cells can be induced by proinflammatory cytokines such as IL-1 β and TNF α , we sought to determine whether VCAM-1 expression will be similarly upregulated in prostate cancer epithelial cells exposed to THP-1 conditioned media. Untreated LNCaP cells, and LNCaP cells treated with LPS or conditioned media from unstimulated THP-1 macrophages did not express detectable levels of VCAM-1 by Western analysis or flow cytometry analysis (Figure 4). In contrast, LNCaP cells exposed to conditioned media from LPS-treated THP-1 macrophages had significantly elevated VCAM-1 expression (Figure 4). Single cell analysis by flow cytometry indicated that the induction of VCAM-1 expression was limited to a subset of treated LNCaP cell (Figure 4b). On average, 11.4 $\pm$ 2.2% of LNCaP cells exposed to conditioned media from LPS-treated THP-1 macrophages had elevated VCAM-1 expression, as compared to less than 1% VCAM-1 expression in control groups (Figure 4c).

3.5. Upregulation of the nuclear estrogen receptor α (ER α) in LNCaP cells treated with LPS-activated THP-1 cells conditioned media

Estrogens have been implicated to be involved in prostate carcinogenesis [21;22]. In particular, ER α is associated with aberrant proliferation, inflammation, and the development of malignancy. Increased expression of ER α has been detected in prostate cancer and premalignant prostatic lesions [23]. To determine if THP-1 macrophage conditioned media affects the expression of ER α in prostate cancer epithelial cells, we quantified nuclear ER α expression in LNCaP cells exposed to THP-1 conditioned media by western analysis (Figure 5). Minimal expression of nuclear ER α was detected in control cells. In contrast, a significantly elevated expression of nuclear ER α was detected in LNCaP cells treated with conditioned media from LPS-stimulated THP-1 cells.

Discussion

Increasing evidence suggests that chronic inflammation plays an important role in prostate carcinogenesis. However to date, it is unclear how chronic inflammation is established in the prostate. Specifically, the relative contribution of activated immune cells such as macrophages, and prostate epithelial cells in perpetuating the inflammatory response that ultimately results in prostate carcinogenesis remains unresolved. Our study focuses on the relationship between macrophages and prostate cancer epithelial cells in sustaining a chronic inflammatory response. In order to distinguish direct inflammatory response elicited by prostate epithelial cells versus inflammatory macrophages, we used an in vitro culture system in which androgen-dependent LNCaP prostate cancer epithelial cells were exposed to conditioned media harvested from LPS-activated THP-1 macrophages. This allows us to evaluate the response of LNCaP cells to the entire collection of soluble mediators produced by activated macrophages. In our study, exposure of LNCaP prostate epithelial cells to conditioned media from LPS-activated THP-1 macrophages triggered significant activation of NF κ B as shown by increased nuclear translocation of NF κ B p65 protein with concomitant degradation of cytosolic I κ B α (Figure 2).

Although the short duration of our experiment cannot mimic the sustained exposure of prostate epithelial cells in a chronically inflamed tissue microenvironment, nevertheless, our finding indicated that exposure of prostate epithelial cells to proinflammatory soluble mediators directly activated NF κ B and induced local production of proinflammatory cytokines TNF α , IL-1 β , and IL-6 in the prostate epithelial cells. Secretion of proinflammatory cytokines by prostate cells likely results in further recruitment and activation of additional immune cells, thus amplifying the proinflammatory response via a positive feedback mechanism. Furthermore, activated macrophages induced prostate epithelial cells to upregulate VCAM-1 and ER α expression, two molecules that are implicated in cancer progression. This study highlights the interactions between inflammatory response and prostate cells during the carcinogenic process and suggests that prostate epithelial cells can play a direct and critical role in propagating a local inflammatory response. Our results also indicate a close interplay between immune cell and prostate epithelial cell responses in both amplifying inflammatory responses and influencing key pathways involved in the carcinogenic process.

Inflammation is a physiologic process mediated by the innate immune system to protect against pathogen infections, environmental insults, and wounding. Chronically inflamed tissues undergo continuous tissue damage and repair, which can eventually lead to sustained and dysregulated cellular proliferation and potentially results in an accumulation of genetic mutations that provides the initiation events in cancer development. Once cancerous lesions develop, a chronically inflamed tumor microenvironment stimulates aberrant angiogenesis, and promotes invasion and migration of tumor cells. The tumor inflammatory microenvironment are infiltrated with multiple subsets of immune cells, including macrophages, neutrophils, eosinophils, dendritic cells, mast cells, and lymphocytes [3]. In particular, we focus on macrophages in this study because macrophages are a major component of immune infiltrates present in the tumor microenvironment and play one of the key roles in chronic inflammation [10;11]. Furthermore, tumor-associated macrophages are involved in promoting tumor progression and metastasis by inhibiting anti-tumor immunity [24].

Recent studies have identified the transcription factor family NF κ B as a key modulator in driving inflammation-induced cancers [12]. NF κ B is activated by inflammatory stimuli. Targets of NF κ B include genes regulating immune response, inflammation, cell proliferation, cell migration, and apoptosis. While normal NF κ B activation is tightly regulated, constitutive activation of NF κ B has been reported in several types of cancer, suggesting aberrant NF κ B activation may play an important role in linking chronic inflammation to the promotion of cancer development [25;26;27]. However, in a chronically inflamed tumor environment, it is difficult to distinguish the whether aberrant NF κ B activation originates from tumor cells or from immune infiltrates. Our results demonstrated that NF κ B can be locally activated in prostate cancer epithelial cells upon stimulation, and induce a significant production of proinflammatory cytokines TNF α , IL-1 β , and IL-6 directly by the prostate epithelial cells. This underscores the direct contribution of prostate epithelial cells during the inflammatory process and their involvement in a positive feedback loop that results in chronic inflammation and tumor proliferation.

We have also identified potential links between prostate inflammation and events culminating in prostate cancer progression. The transition from premalignant prostate lesions to adenocarcinoma and eventually metastatic tumors is a multistep process. Among them, immune evasion is one mechanism through which tumor escapes T cell-mediated immune attacks. Expression of the adhesion molecule VCAM-1 in tumor has been shown to result in decreased trafficking of tumor-specific T cells in the tumor microenvironment, leading to tumor escape from anti-tumor immunity [19]. Furthermore, circulating soluble VCAM-1 in blood is a biomarker that predicts prostate cancer relapse after radical prostatectomy in patients [20]. Since VCAM-1 expression in endothelial cells can be induced by proinflammatory

cytokines such as IL-1 β and TNF α , we sought to determine if VCAM-1 expression was similarly upregulated in prostate epithelial cells upon exposure to activated macrophages. Significant increase in VCAM-1 expression was detected in LNCaP cells treated with LPS-activated THP-1 conditioned media (Figure 4). Flow cytometry analysis indicated that VCAM-1 expression was induced in ~12% of treated LNCaP cells. Our result suggests this subset of VCAM-1 positive prostate cancer cells may have a selective advantage and are more likely to evade anti-tumor immunity. Other mechanisms implicated in prostate cancer progression include estrogen regulation [21]. Estrogen plays a role in regulating the growth, development, and function of the prostate along with androgens. Estrogen regulation has been implicated as one of the risk factors in association with the development of prostate cancer [28;29]. The action of estrogen is mediated by nuclear estrogen receptors. In particular, expression of ER α is associated with aberrant proliferation, inflammation, and the development of malignancy. Moreover, the extent of detectable ER α expression correlates with the different stages of prostate cancer, from premalignant lesions to adenocarcinoma [23]. In our study, we observed an increased expression of nuclear ER α in LNCaP cells upon stimulation with activated macrophage conditioned media (Figure 5). Our results indicate that exposure of prostate epithelial cells to proinflammatory soluble mediators is one potential mechanism that upregulate ER α expression in prostate epithelial cells, and may in part contribute to prostate cancer progression.

Taken together, we showed that prostate cancer epithelial cells can directly contribute to the propagation of the proinflammatory response upon encountering activated macrophages. This likely establishes a positive feedback mechanism that can further recruit and activate immune cells, and sustain a proinflammatory response in the prostate microenvironment. Furthermore, exposure of prostate cancer epithelial cells to soluble inflammatory mediators may promote tumor progression by increasing their potential for immune evasion and tumor metastasis.

Acknowledgments

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Figure 1. Low dose LPS induces the production of proinflammatory cytokines in THP-1 macrophages. Differentiated THP-1 macrophages were left untreated or were activated with 10 ng/ml LPS for 48 h. Proinflammatory cytokines TNF α (A), IL-1 β (B), and IL-6 (C) secreted into culture supernatants were quantitated by cytokine-specific ELISA. Data represents mean $\pm$ SEM, n=3. * p $\leq$ 0.0001.

Figure 2.

Nuclear translocation of NFκB p65 and degradation of cytosolic IκBα in LNCaP cells treated with LPS-activated THP-1 macrophages conditioned media. LNCaP cells were treated with 10 ng/ml LPS, conditioned media from untreated THP-1 macrophages (THP untx), conditioned media from LPS-activated THP-1 macrophages (THP LPS), or left untreated (untx) for 3 h. Nuclear and cytosolic protein extracts were isolated from treated LNCaP cells. Nuclear NFκB p65 and cytosolic IκBα were detected by Western analysis (A). Equal protein loading was confirmed using β-actin. Mean densitometry of NFκB p65 (B) and IκBα (C) normalized to β-actin ± SEM are shown (n=3). * p<0.001 versus untreated; + p<0.01 versus THP-untreated.

Figure 3.

Production of proinflammatory cytokines in LNCaP cells treated with LPS-activated THP-1 cells conditioned media. LNCaP cells were treated with 10 ng/ml LPS, conditioned media from untreated THP-1 macrophages (THP untx), conditioned media from LPS-activated THP-1 macrophages (THP LPS), or left untreated (untx) for 24 h. Cytosolic protein extracts were isolated from equal number of treated LNCaP cells. Intracellular production of the proinflammatory cytokines TNF α (A), IL-1 β (B), and IL-6 (C) were quantitated by cytokine-specific ELISA. Data represents mean $\pm$ SEM, n=3. * $p \leq 0.01$ versus untreated.

Figure 4.

Induction of VCAM-1 expression in LNCaP cells treated with LPS-activated THP-1 cells conditioned media. LNCaP cells were treated with 10 ng/ml LPS, conditioned media from untreated THP-1 macrophages (THP untx), conditioned media from LPS-activated THP-1 macrophages (THP LPS), or left untreated (untx) for 24 h. (A) Cell lysates were prepared for Western analysis of VCAM-1 expression (n=3). Equal protein loading was confirmed using β -actin. (B) Representative flow cytometry analyses of surface VCAM-1 expression in treated LNCaP cells. Gated region denotes the percentage of VCAM-1-positive cells. (C) Averaged percentage of LNCaP cells with surface VCAM-1 expression based on flow cytometry analyses ($\pm$ SEM, n=3, * $p < 0.01$ versus untreated).

Figure 5.

Induction of nuclear ERα in LNCaP cells treated with LPS-activated THP-1 cells conditioned media. LNCaP cells were treated with 10 ng/ml LPS, conditioned media from untreated THP-1 macrophages (THP untx), conditioned media from LPS-activated THP-1 macrophages (THP LPS), or left untreated (untx) for 3 h. Nuclear extracts were isolated from treated LNCaP cells. (A) Nuclear ERα was detected by Western analysis. Equal protein loading was confirmed using β-actin. (B) Mean densitometry of ERα normalized to β-actin ± SEM (n=3). * p<0.01 versus untreated.

Table 1

mRNA expression of proinflammatory cytokines and nitric oxide in THP-1 macrophages treated with low dose LPS

Gene of interest	Normalized fold-change *
TNF α	167.5 +/- 49.5
IL-1 β	12.5 +/- 0.5
IL-6	234.0 +/- 45.0
iNOS	1.02 +/- 0.04

* Data represents mean fold-change +/- SEM of normalized gene expression in LPS-treated THP-1 macrophages divided by normalized untreated THP-1 macrophages in two biological replicates.