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Prostate Inflammation and Its Potential Impact on Prostate Cancer: A Current Review

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Abstract Recent studies have identified a role for inflammation in the development and progression of several cancers, such as liver, stomach and the large intestine. Data from several studies has shown correlations between soluble inflammatory mediators, such as cytokines, chemokines and growth factors. However, a direct relationship between inflammation and prostate cancer has yet to be identified. Two major hurdles currently exist which limit the study of this relationship are first that animal models available for studying prostate inflammation are lmited, and secondly that relatively little is known about the inflammatory response in the prostate. Here we first review the data demonstrating a correlation between inflammation and prostate cancer as well as review what is currently known about the inflammatory response in the prostate and the impact this inflammation has on the prostate tissue. J. Cell. Biochem. 103: 1344–1353, 2008.

Key words: prostate; prostatitis; prostate cancer; inflammation; mouse model; POET-3

Prostate cancer is the most frequently diagnosed cancer in males, and it is estimated more than 218,000 new cases will be diagnosed in 2007 [American Cancer Society, 2007]. The risk for developing prostate cancer increases with age and for men between the ages of 60 and 69, an estimated 1 in 14 will be diagnosed with this disease. The pathogenesis of prostate cancer has been linked to both genetic and environmental factors, and growing evidence suggests a role for chronic inflammation in prostate cancer [De Marzo et al., 1999; Nelson et al., 2004]. Indeed, a causative role for inflammation in several other cancers has already been established. It is estimated that around 20% of all human cancers including cancers of the stomach, liver, and large intestine arise from chronic inflammation [Ames et al., 1995]. Several studies have correlated the presence of inflammatory cytokines, chemokines, and growth factors with an increased risk for developing prostate cancer. These correlative data have lead to the hypothesis that chronic inflammation plays a role in the development and progression of prostate cancer. However, a direct relationship between inflammation and prostate cancer has yet to be demonstrated.

The lack of data supporting a direct impact of inflammation on prostate cancer can be attributed to several limitations in the field. First, the majority of diagnosed cases of chronic prostate inflammation in humans are of unknown etiology, a fact that has made development of relevant animal models difficult. Secondly, at this time there are no animal models that allow the inflammatory response in the prostate to be studied in an antigen specific system. Development of antigen specific animal models is necessary in order to directly address the impact of inflammation on the prostate, and its regulation. Thirdly, lack of animal models has contributed to the fact that the impact of

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inflammation on the normal prostate is an understudied area of research. Clearly, in order to study the impact of inflammation on prostate cancer, prostate inflammation itself must be better understood.

The prostate is an organ that matures at the onset of puberty when the presence of hormones, such as androgens, induce the expression of prostate specific genes and new self-antigens. Androgens, in addition to inducing the development of the prostate, can dampen immune responses in the prostate [Mercader et al., 2001; Roden et al., 2004]. These factors make the prostate a unique and immunologically complex organ, and necessitate further study of the inflammatory and immune responses to more clearly understand their roles in the development of prostate cancer.

The immune response in the prostate and the impact of an inflammatory environment on the prostate are just now beginning to be studied. This manuscript will provide an overview of the data correlating prostate inflammation and prostate cancer, and review what is known about prostate specific inflammation in both a benign and tumor setting. It will also address the impact that inflammation has on prostate tissue.

A CAUSAL RELATIONSHIP BETWEEN INFLAMMATION AND PROSTATE CANCER

Studies have shown a correlation between inflammatory cytokines, chemokines and an increased risk for developing prostate cancer. However, a direct relationship between inflammation and prostate cancer has yet to be conclusively demonstrated. As stated above, this is largely due to limited knowledge about the prostate specific inflammatory response. Additional work is required to identify the mechanisms by which immune cells infiltrate prostate tissue, and their impact on prostate epithelial and stromal cells.

Much of the data supporting the hypothesis that an inflammatory environment plays a role in development and progression of prostate cancer was generated with cell lines. These data have implicated immune cell mediated production of proinflammatory cytokines, chemokines, and matrix metalloproteinases (MMPs) in prostate cancer. In some cases, there are clinical data to support these observations, but the full impact of these inflammatory mediators on the prostate has yet to be determined. This section will outline what is known about inflammatory mediators and prostate cancer.

Cytokines, Chemokines and Prostate Cancer

Interleukin-1 (IL-1), is a proinflammatory cytokine, and has been shown to promote the growth and progression of several solid tumors [Voronov et al., 2003]. IL-1β is necessary but not sufficient for metastasis of both B16 melanoma cells and prostate cancer cells [Voronov et al., 2003]. In a model of mammary carcinoma, IL-1α was necessary for angiogenesis [Voronov et al., 2003]. These data indicate the effect of IL-1 on tumor cells in vivo is likely dependent upon the type of the tumor cell, and may depend on both intrinsic qualities of the tumor cells themselves as well as tissue specific factors.

Another proinflammatory cytokine which may be involved in the cross-talk between inflammatory cells and prostate tumor cells is IL-6. In addition to its involvement during normal immune responses, IL-6 can influence malignant processes such as apoptosis and angiogenesis [Culig et al., 2005]. Another important function of this cytokine in the progression of tumors is the ability to regulate cell growth. IL-6 enhances proliferation and acts as a survival signal for many malignant cell types including prostate tumor cell lines [De Marzo et al., 1999; Nelson et al., 2004]. Clinically, elevated serum IL-6 levels are found in patients with metastatic disease, and are associated with poor prognosis in patients with various cancers, including prostate cancer [Michalaki et al., 2004].

One controversy concerning the role of IL-6 and prostate cancer comes from studies done using either androgen dependent or androgen independent prostate cell lines. Chung et al. [1999] have demonstrated androgen independent prostate cancer cell lines secrete IL-6, whereas androgen dependent cell lines such as LNCaP cells do not [Chung et al., 1999]. In this same study, IL-6 induced proliferation of prostate cancer cell lines and was dependent upon the state of androgen responsiveness [Chung et al., 1999]. In contrast, Siegall et al. [1990] found that LNCaP cells did produce IL-6 [Siegall et al., 1990]. This controversy highlights the importance of both studying primary human tissue and developing new in vivo models to study the impact of IL-6 and other

inflammatory cytokines on both healthy and transformed prostate cells.

IL-17 is a proinflammatory cytokine secreted by a subset of activated CD4 T cells. IL-17 induces macrophages to secrete tumor necrosis factor alpha (TNF-α) and IL-1β, and can stimulate stromal cells to secrete inflammatory cytokines, chemokines, and growth factors [Fossiez et al., 1996; Jovanovic et al., 1998]. In addition, IL-17 has been shown to be involved in angiogenesis and in vivo growth of tumor cells. IL-17 promotes migration of endothelial cells and induces fibroblasts to up-regulate proangiogenic factors, such as vascular endothelial growth factor (VEGF), macrophage inflammatory protein-2 (MIP-2), prostaglandins, and nitric oxide (NO) [Numasaki et al., 2003]. Given the potent proinflammatory nature of IL-17, it is not surprising that a study by Steiner et al. [2003] found an increase in IL-17 mRNA in 58% human malignant prostate tissue [Steiner et al., 2003]. This same study reported that treatment of rat prostate stromal cells and prostate cancer cell lines with IL-17 increased mRNA and protein expression of IL-6 and IL-8 [Steiner et al., 2003]. These data indicate IL-17 may be involved in increasing prostate tumor cell growth and metastasis by either directly acting on the prostate cells, or indirectly by increasing the level of inflammatory cytokines and growth factors locally in the prostate.

In humans, IL-8 is an inflammatory chemokine that recruits neutrophils to the site of injury during a normal immune response. IL-8 can also promote the growth of tumor cells, and has been shown to influence the progression of solid tumors such as prostate cancer, largely due to its ability to induce expression of MMPs. When secreted, MMPs break down components of the extra-cellular matrix, a process that then leads to tumor cell extravasation, and increased rates of metastasis [Thomas et al., 2001]. IL-8 has been shown to regulate the expression of MMPs, such as MMP-9, and thus IL-8 can promote angiogenesis and metastasis of tumors [Wang et al., 1990; Inoue et al., 2000]. In support of a proangiogenic role of IL-8, numerous studies have demonstrated correlations between MMPs, IL-8, and prostate cancer. Increased levels of IL-8 are associated with higher Gleason scores, and with metastatic disease [Uehara et al., 2005]. Furthermore, elevated levels of MMP-2, and MMP-9 are found to correlate with both prostate cancer metastasis and Gleason score [Kuniyasu et al., 2000]. These data indicate higher levels of IL-8 lead to increased MMP-9 expression and in turn may directly increase tumor grade and metastasis in prostate cancer patients. Interestingly, both inflammatory cells and prostate cancer cells can produce IL-8 [Ferrer et al., 1998; Inoue et al., 2000]. Current evidence suggests that an inflammatory environment not only directly impacts growth and spread of prostate cancer, but also effects the ability of tumor cells themselves to modify their environment. However, it is important to note that these data are only correlative and to determine the causal relationship between IL-8, MMP-9, and prostate cancer, further in vivo studies using animal models, as well as ex vivo experiments using primary human tissue are required.

TNF-α is another proinflammatory cytokine thought to play a role in the growth of solid tumors. Elevated serum levels of both TNF-α and IL-6 have been shown to correlate with advanced disease, and decreased survival in prostate cancer patients [Michalaki et al., 2004]. Additionally, both in vitro and in vivo data have demonstrated TNF-α can up-regulate αvβ6 expression, and as a result increase MMP-9 expression [Thomas et al., 2001; Scott et al., 2004]. MMP-9 expression results in the extracellular matrix degradation and tumor progression and metastasis [Thomas et al., 2001; Ahmed et al., 2002; Scott et al., 2004]. Additional mechanisms for TNF-α mediated tumor promotion are further discussed in a recent review by Balkwill [2006].

Transforming growth factor beta (TGF-β) is a multi-functional cytokine that differentially regulates the growth and survival of transformed and non-transformed cells. TGF-β inhibits the growth and proliferation of non-transformed epithelial cells, endothelial cells, as well as hematopoietic cells [Elliott and Blobe, 2005]. However, TGF-β increases survival and proliferation of transformed cells, including transformed prostate epithelial cells, and is found at elevated levels in the serum of human prostate cancer patients with metastatic disease [Park et al., 2003; Lu et al., 2004]. These data suggest a role for TGF-β in the progression and metastasis of prostate cancer. Loss of TGF-β types I and II receptors on transformed human prostate epithelial cells correlates inversely with tumor grade, and may allow escape from TGF-β mediated growth regulation [Kim et al., 1996]. Another mechanism that may explain the tumor

promoting functions of TGF-β in malignant cells is loss of proper receptor function. This hypothesis was tested through the adoptive transfer of pre-malignant rat prostate epithelial cells lines (NRP-152). NRP-152 cells were retrovirally transduced with a dominant negative mutant type II TGF-β receptor, and injected into nude mice [Tang et al., 1999]. Roughly one-third of the injected mice developed carcinomas, compared with none of the control animals. These data indicate that the loss of responsiveness to TGF-β growth signals is involved in the transformation of prostate epithelial cells, and enables some tumor cells to evade normal growth regulation by this cytokine.

Human prostate epithelial cells can secrete TGF-β [Lu et al., 2004]. Furthermore, TGF-β secreted from these cells can activate NF-kB (a major transcription factor involved in promoting cell growth and the production of proinflammatory cytokines) and directly increase tumor cell survival [Lu et al., 2004]. TGF-β mediated activation of NF-kB may explain the ability of this cytokine to regulate the expression of other inflammatory cytokines and chemokines in prostate cancer cells, as TGF-β signaling leads to up-regulation of IL-8 expression by prostate cancer cells [Lu and Dong, 2006]. In addition to secreting TGF-β, prostate-specific antigen (PSA), a serine protease secreted by prostate epithelial cells, can cleave latent TGF-β into the active form [Dallas et al., 2005].

In addition to proinflammatory cytokines, chemokines are also involved in the growth of transformed cells. Prostate cancer cell lines have been found to produce high levels of macrophage chemotactic protein (MCP-1), which modulates proliferation and invasiveness of prostate cancer cells through chemokine receptor 2 (CCR2) and Phosphatidylinositol 3 kinase (PI3 kinase) [Lu et al., 2006]. Furthermore, the expression of CCR2 by prostate cancer cell lines has been associated with metastatic potential, and correlated with Gleason score [Lu et al., 2007]. These data suggest that MCP-1 acts through CCR2 to promote metastasis. However, there are two possible explanations for the correlation between MCP-1, CCR2, and progression of prostate cancer. First, MCP-1 and CCR2 may directly induce metastasis of prostate cancer cells. Secondly, their metastatic effects may be secondary to the recruitment of macrophages and the inflammatory mediators secreted by these cells. The distinction between these two possibilities and the role of macrophages in prostate cancer metastasis in vivo requires further investigation.

In addition to a role in metastasis, macrophages have been shown to be involved in inducing hormone resistance in prostate cancer cell lines. Zhu et al. [2006] demonstrated macrophages and prostate cancer cells interact via VCAM-1 expression on the prostate cancer cells [Zhu et al., 2006]. This interaction was shown to induce macrophage activation and secretion of proinflammatory cytokines such as IL-1β, which led to increased resistance to androgen antagonists [Zhu et al., 2006].

Summary

Among the possible interpretations for these correlative data, one may conclude that the presence of inflammatory chemokines and cytokines in the prostate indicates a causative role for these mediators in the development of prostate cancer. Secondly, one may also conclude that the presence of these inflammatory mediators is the result of the tumor itself being inherently inflammatory. This second point may seem unlikely given the fact that a tumor environment is often seen as an immunosuppressive environment. However, prostate tumor cells can secrete proinflammatory cytokines, an event that may both result in and result from constitutive NF-kB activation. The ability of tumor cells to modulate such an important inflammatory pathway suggests tumors may benefit from an inflammatory environment. The specific role of inflammatory cells and their secreted products in the prostate is an area which demands further study. The overwhelming question that remains to be answered is whether or not inflammatory cytokines, chemokines and leukocytes are present as a result of the cancer, or are the result of transformation events which precede the infiltration of inflammatory cells. Until future studies aimed at clarifying the role of inflammation and prostate cancer are completed these questions will remain unclear.

CHRONIC INFLAMMATION AND PROSTATE CANCER: PROSTATITIS

Chronic inflammation is linked to prostate cancer. Prostatits affects 3–16% of men in the US, and is the most frequently seen outpatient condition in urologic practice in men younger

than 50 years of age [Collins et al., 1998]. Many studies concerning prostate inflammation and the risk for prostate cancer have looked at the relationship between men who develop chronic prostate inflammation, prostatitis, and those who go on to develop prostate cancer. One such study examined prostate needle biopsy specimens from 177 men with abnormal clinical results. From this group, 144 had chronic inflammation. Over the next 5 years, 20% of the 144 patients with chronic inflammation were diagnosed with chronic inflammation and adenocarcinomas, and 6% of the chronic inflammatory patients were diagnosed with high-grade prostatic intraepithelial neoplasia. In contrast, of the 33 patients who did not have chronic prostatic inflammation, only 6% of the patients were found to have newly diagnosed adenocarcinomas [MacLennan et al., 2006]. These data demonstrate a history of chronic inflammation is associated with an increased risk for prostate cancer. These data were supported by a meta-analysis of data published prior to 2000, that found men with a history of prostatitis had an increased risk for developing prostate cancer [Dennis et al., 2002]. These data did not examine the type of prostatitis associated with an increased risk, but found that a history of syphilis and gonorrhea also increased the risk for developing prostate cancer [Dennis et al., 2002]. Furthermore this study found that a history of sexually transmitted diseases correlates with prostate cancer, and implicates chronic inflammation in the promotion of prostate cancer. It is important to note, however, that a history of STDs does not always lead to progression or development of cancer. Therefore additional studies are necessary to identify specific inflammatory factors involved in transformation of prostate cells.

The strongest evidence that inflammation can impact the development of prostate cancer comes from recent work done by Elkahwaji et al. [2007] using a murine model of chronic bacterial prostatitis induced by *E. coli* infection. After 12 weeks of infection, a time at which chronic inflammation was established, both prostate hyperplasia and dysplasia were seen. Additionally, prostates from these mice had positive nuclear staining for 8-hydroxy-2′-deoxyguanosine, a marker for DNA damage by oxidative stress [Elkahwaji et al., 2007]. This study demonstrates that an inflammatory environment can directly modify the prostate by inducing DNA damage and induce morphological changes leading to transformation.

Summary

These studies illustrate the point that both epidemiological and laboratory based studies have established a link between chronic inflammation and an increased risk in prostate cancer. In vivo studies have begun to study the impact of inflammation on prostate tissues. However, the impact of an inflammatory environment in the prostate, and the mechanisms that lead to the development of prostate cancer are not yet clear.

Prostate Infiltrating Leukocytes

Early studies looking at the prostate specific immune response examined the role of androgen on leukocyte function, and helped to establish the prostate as an organ with a unique immunobiology [Mercader et al., 2001; Roden et al., 2004]. The affects of androgen ablation can be seen both locally in the prostate and in the periphery as well. After androgen ablation, large numbers of T cells migrate into the prostate, and in peripheral lymphoid organs T cell numbers increased [Mercader et al., 2001; Roden et al., 2004]. Furthermore, when tested in vitro, T cells isolated from the peripheral lymphoid organs of mice undergoing androgen ablation have a lower activation threshold [Roden et al., 2004]. Two possible interpretations of these data are, either that androgen is a negative regulator of inflammation, or that increased T cell migration and activation is a result of increased apoptosis of prostate epithelial cells after androgen withdrawal. In situations where massive amounts of apoptotic material are found, such as in the case of prostate involution after androgen withdrawal, normally inhibitory apoptotic signals may become inflammatory. The latter explanation is supported by data demonstrating that dendritic cells pulsed with apoptotic tumor cells can prime anti-tumor responses in vivo [Ronchetti et al., 1999]. Importantly, this study shows that apoptotic cells are immunogenic in vivo, and may be able to induce an inflammatory response. The nature of the antigen released from apoptotic prostate epithelial cells may also be involved. Indeed, elevated numbers of activated macrophages and dendritic cells have

been found in human prostate tissue obtained following androgen ablation [Mercader et al., 2001]. These data suggest that androgen withdrawal may increase activation of prostate specific T cells by inducing apoptosis of prostate epithelial cells, increasing the amount of prostate antigen presented to them by APCs. One question that remains, however, is the function of infiltrating T cells in the prostate. Mercader et al. [2001] went on to show that T cells infiltrating the prostate have a restricted Vβ gene usage; however, their function was not examined [Mercader et al., 2001]. This evidence indicates T cells infiltrating the prostate may be auto-reactive and have the ability to induce prostate inflammation by damaging prostate epithelial cells. Future studies aimed at identifying the role of T cells in the prostate are necessary to fully determine the impact of androgen withdrawal on the prostate.

Because there are a limited number of available mouse models of prostatitis, much of the data concerning the immune response in the prostate has been generated from tissue samples taken either through autopsy or biopsy. One study looking at autopsy samples found that normal prostate tissue contains leukocytes. These cells were largely composed of T cells, with higher numbers located in the stroma than in the epithelium [Bostwick et al., 2003]. Interestingly, CD4 T cells were pre-dominately found in the stroma of the prostate, whereas CD8 T cells were more commonly found in the epithelium. In a separate study, biopsy samples taken from prostate cancer patients demonstrated that increased CD4 T cell infiltrate is associated with poor survival rates. However, the activation of these cells was not examined [McArdle et al., 2004]. Determining the phenotype of infiltrating lymphocytes will aid in understanding their impact on the prostate. While these data are directly relevant, they do not address the function of infiltrating cells or the impact they may have on the prostate tissue itself. A more thorough phenotype analysis of lymphocytes isolated from human tissue is needed in order to answer questions about their function in prostate inflammation. These data illustrate an important point: it is difficult to determine the role leukocytes in the prostate without testable animal models.

In vitro studies using primary peripheral blood mononuclear cells (PBMCs) from chronic prostatitis patients have begun to identify the role of infiltrating lymphocytes in prostatitc disease. Auto-reactive lymphocytes able to produce IFNγ in response to prostate auto-antigens have been found in prostate tissue samples taken from patients with chronic non-infectious prostatitis [Motrich et al., 2005]. In fact, 34% of these patients had IFNγ positive lymphocytes specific for PSA [Motrich et al., 2005]. The role of these auto-reactive lymphocytes in the inflammatory process was not determined, nor was the impact of these cells on the prostate. In order to identify the role of infiltrating leukocytes and their secreted soluble factors on the prostate, animal models are needed that allow focused questions in an antigen specific system of prostate inflammation to be asked.

One important issue concerning the presence of leukocytes in the prostate is determining what factors influence their migration into the tissue. The migration of leukocytes into the prostate may be influenced by the secretion of proinflammatory chemokines or other cytokines by either resident lymphocytes or prostate epithelial cells. This hypothesis is supported by data showing that prostate epithelial cells are directly involved in the recruitment of inflammatory cells [Wang et al., 2004]. Increased COX-2 production by prostate epithelial cells in benign prostatic inflammation was associated with the presence of infiltrating T cells and macrophages [Wang et al., 2004]. COX-2 staining of epithelium was associated with large numbers of CD3 cells; 21.6% of glands with T cell inflammation expressed COX-2 as opposed to 0.3% that expressed COX-2 with no T cells present. Additionally, 29.4% of glands with macrophage inflammation had epithelial staining for COX-2 [Wang et al., 2004]. The same group extended this association to transformed prostate epithelial cells. Among transformed prostate epithelial cells, those expressing COX-2 also had high numbers of T cells and macrophages [Wang et al., 2005]. These data further support a role for inflammation in prostate cancer as COX-2 expression was also found to be associated with increased cell proliferation and expression of the anti-apoptotic molecule, Bcl-2 [Wang et al., 2004]. Thus COX-2 produced by prostate epithelial cells may contribute toward inflammation induced transformation of prostate epithelial cells, both by inducing migration of T cells and macrophages into the prostate, but by also contributing to uncontrolled cell proliferation, and increased survival.

Much of the data concerning prostate infiltrating leukocytes does not address the function or impact of these cells on prostate cancer. However, a few studies using murine models of prostate cancer have begun to investigate this issue. Anderson et al. [2007] demonstrated tumor specific CD8 T cells first encounter antigen in lymph nodes, where they proliferate briefly before undergoing apoptosis [Anderson et al., 2007]. This study also showed that a subset of the tumor specific CD8 T cells trafficked to the prostate, and were found to have an activated phenotype [Anderson et al., 2007]. Despite the expression of activation markers, T cells isolated from prostate tissues were tolerized to tumor specific antigen [Anderson et al., 2007]. The mechanism of tolerance induction was not identified, and may be attributed to the presence of CD4 T regulatory cells (Tregs).

The presence of Tregs in both prostate tissue and peripheral blood has been identified in prostate cancer patients. One recent paper examining prostate tumor tissue taken from human patients reported an increased number of Tregs compared to benign prostate tissue in the same individual [Miller et al., 2006]. These data present a confusing situation, if Tregs are present in increased levels in transformed prostate tissue, it seems that inflammation and T cell activation would be dampened locally in the transformed prostate tissue. But contrary to this idea, as discussed above, transformed prostate tissue has been shown to be associated with inflammatory mediators and increased levels of T cells and macrophages. Two potential explanations for these data are that the increased level of Tregs present in the transformed tissue may be a function of inflammatory chemokines that attract not only Tregs but also effector T cells. Secondly, the local presence of inflammatory cytokines and chemokines produced by macrophages and T cells that are activated in the periphery and later migrate to the prostate may decrease the ability of Tregs to suppress inflammation in the prostate. Indeed cytokines such as IL-12, IL-4, and IL-21 have all been shown to modulate the ability of Tregs to suppress effector T cells [Peluso et al., 2007]. In addition to elevated levels of Tregs in the prostate, higher levels of Tregs have also been found in the peripheral blood of prostate cancer patients. Importantly, functional data indicated that Tregs retained suppressive function in vitro [Miller et al., 2006]. These data indicate that the function of Tregs may be influenced by the local environment and further support the hypothesis that regulation can be modified by local inflammatory signals.

In addition to the presence of macrophages and T cells in the prostate, mast cells have also been identified in prostate tumors. Mast cells have been implicated in the growth of tumors by increasing tumor vascularization and metastasis. In certain cancers, mast cell numbers positively correlate with numbers of blood vessels, and indicate a role for mast cells in growth and metastasis of prostate tumors [Yano et al., 1999]. Prostate tissue obtained from patients with prostatic adenocarcinoma showed an increased number of mast cells within the tumor correlated with the Gleason score [Sari et al., 1999]. Thus, based on these data mast cells may impact tumor growth and metastasis by increasing tumor vascularization.

Correlative data indicate that proinflammatory cytokines and other factors increase prostate tumor growth and progression. However, it is also possible that the growth of the tumor may also impact the ability of inflammatory cells to infiltrate the tissue. Indeed a barrier function for tumor vasculature has been suggested due to decreased levels of adhesion molecules on endothelial cells in both breast and renal cell carcinomas. This finding was extended to prostate tumor neovasculature by demonstrating impaired extravasation of lymphocytes into prostate tumors due to loss of adhesion molecules on tumor blood vessels [Fedida et al., 2007]. The concept that the tumor vasculature acts as a barrier to infiltrating lymphocytes is in apparent contradiction with data from McArdle et al. [2004] who demonstrated that prostate tumors had elevated numbers of CD4 and CD8 T cells. Furthermore, McArdle et al. found no correlation between Gleason score and CD4 or CD8 T cell counts, and Fedida et al. used tumor tissue taken from patients with a range of Gleason scores. This evidence suggests that the ability of lymphocytes to enter the prostate is influenced by more than just the stage of the tumor itself.

These studies summarize what is known about inflammatory cells in the prostate, but are largely limited to identification of these cells in the prostate. In order to determine the role of inflammation in the development of prostate

cancer, the immunobiology of the prostate inflammatory response must first studied using in vivo model systems. Currently, few animal models exist that allow prostate inflammation to be studied in vivo in a controlled and repeatable model system, a fact that underlines the need for development of improved animal models. One model system that allows the study of inflammatory cells in a tumor setting is the TRAMP model. This mouse was generated with the SV40 tumor antigen targeted to the prostate epithelium by the rat probasin promoter [Gingrich et al., 1996]. The TRAMP mouse is a model of metastatic prostate cancer that shares some similarities with human disease in that loss of E-cadherin expression and some metastasis to bone [Gingrich et al., 1996]. However, while this is a model system in which cancer is present, no independent inflammation is present. This illustrates one of the limitations in the field, the current lack of model systems which allow the study of both inflammation and prostate cancer in the same model system.

In order to ask direct questions about the role of lymphocytes in prostate inflammation and their impact on prostate tissue itself, our laboratory has generated and characterized a novel transgenic mouse model of inducible prostate specific inflammation, that is, antigen specific. This mouse model, the prostate ovalbumin expressing transgenic -3 (POET-3) model has ovalbumin (OVA) expressed as a membrane antigen on the surface of prostate epithelial cells. Prostate specific inflammation can be induced in the POET-3 mouse by adoptively transferring pre-activated OVA-specific OT-I T cells intravenously (i.v.). This induced inflammation results in targeted but limited destruction of the prostate epithelial cells by antigen specific CD8 T cells. Inflammation can result in damage to epithelial cells, and as a result may cause loss of tolerance to normal prostate antigens, resulting in an auto-immune reaction. Thus these data suggest the POET-3 model system is a clinically relevant model for studying prostate inflammation. Another advantage of this model is that the OVA containing transgene is not detected in the thymus, which indicates that regulation of prostate inflammation may parallel the regulation in the human prostate, an idea our laboratory is currently investigating.

In addition to investigating prostate inflammation and its regulation, our laboratory has generated a model system in which we can routinely induce inflammation in an established tumor environment. To create these mice, High Myc mice over-expressing the Myc transcription factor were crossed with our POET-3 mice. High Myc mice have been engineered to have human c-Myc driven from a prostate specific promoter containing two androgen responsive elements, resulting in high levels of Myc expression in the prostate [Ellwood-Yen et al., 2003]. Importantly, elevated levels of Myc have been found in numerous human cancers, including prostate cancer and provide a clinical relevance for this model system [Ellwood-Yen et al., 2003]. Our POET-3 High Myc mice provide a model system in which tumors develop in the prostate with age, and in which inflammation can be induced by adoptive transfer of antigen specific CD8 T cells. Not only does this system provide an existing tumor environment, but it is one in which we can induce chronic inflammation. Thus, these mice provide a unique model system to study the impact of inflammation in prostate cancer.

Developing improved model systems will help to identify the impact inflammatory cells, cytokines and damaging oxidative factors have on both healthy and transformed prostate tissue. Certainly, evidence from other cancer models has demonstrated the tumor-promoting role of proinflammatory cytokines, and the damaging effects of reactive oxygen and nitrogen species generated during inflammation. By addressing the impact of inflammation on the prostate, the role of inflammation on the progression of prostate cancer can be examined.

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