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Prostate cancer immunology – an update for Urologists

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A better understanding of the immune processes in the pathogenesis and progression of prostate cancer (CaP) may point the way towards improved treatment modalities. The challenge is to amplify immune responses to combat tumour escape mechanisms. Infection and inflammation may have a role in prostate carcinogenesis, including the newly discovered xenotropic murine leukaemia virus (XMRV). These inflammatory states damage defence mechanisms and induce a high proliferative state favouring further mutation and impaired immune surveillance. With this knowledge we are able to explore the use of immunotherapy to rejuvenate the immune system in combating CaP. Recently Sipuleucel-T, an immunotherapeutic agent for metastatic androgen independent CaP, has resulted in improved survival and might be the first immunotherapeutic agent to obtain approval for CaP treatment. This short review will focus on the growing body of evidence suggesting an immunity-based link between CaP and inflammation and infection.

What's known on the subject? and What does the study add?
It is not commonly appreciated that there is a complex interplay between the immune system, the normal prostate, and the development and progression of prostate cancer. This interaction is finely balanced and is now giving clues to novel treatment approaches. At least one new immunotherapy treatment for prostate cancer is now approved in the US and other approaches are under investigation.

INTRODUCTION

Prostate cancer (CaP) is the most prevalent non-skin malignancy in males in the western world. In the United States it is estimated that 192 280 men were newly diagnosed with CaP and 27 360 died from the disease in 2009 [1]. The purpose of this article is to concisely review the growing body of evidence suggesting an immunity-based link between CaP and inflammation and infection.

POSSIBLE LINK TO CARCINOGENESIS AND TUMOUR PROGRESSION

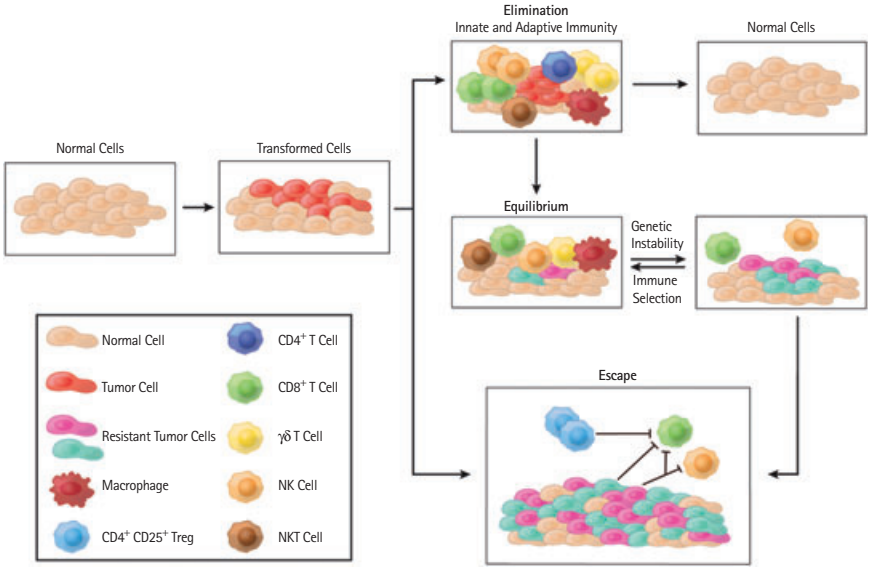
Prostatitis can be associated with infection (acute or chronic bacterial infections) or inflammation with no evidence of infection (abacterial prostatitis/chronic pelvic pain syndrome). A meta-analysis conducted on case-control studies from 1966 to 2000 demonstrated an association between a history of prostatitis and the future risk of developing CaP (odds ratio 1.8, 95% confidence interval 1.1 to 3.0) [2]. It was also observed that there was an increased relative risk, in men with a history of sexually transmitted infections such as syphilis (odds ratio 2.3) and gonorrhoea (odds ratio 1.3). Even though this study is limited by recall bias, it supports a plausible association between CaP and prostatic inflammation.

Inflammation in the prostate could arise after infection, epithelial cellular injury due to urinary reflux resulting in chemical or physical injury, hormonal variations or dietary factors. This can progress to tumourigenesis through a number of mechanisms. The production of free radicals generated by the inflammatory cells in response to initial insult results in DNA damage. Cytokines secreted by activated inflammatory cells are capable of enhancing cell proliferation and facilitating angiogenesis for tumour growth. Cellular proliferation in response to injury increases the cell population at risk of further mutations.

Histological sections of prostate tissue often show evidence of inflammatory infiltration and proliferative inflammatory atrophy (PIA). PIA is a term used to describe tissue with epithelial atrophy, low apoptotic index and increased proliferative index [3]. PIA develops due to the above causes of inflammation and, in its high proliferative state, may accrue a number of mutations, such as GSTP1, to progress to high-grade prostatic intraepithelial neoplasia [4].

Although viruses are often present in prostate tissue, their mechanism of causing an inflammatory response is not fully understood [5]. Viruses such as human papillomavirus, cytomegalovirus and human herpes simplex virus (type 2 and 8) have been detected in the prostate. Recently xenotropic murine leukaemia virus (XMRV), a gammaretrovirus previously found in animals, has been isolated from human CaP tissue. One study showed that it was present in 40% of patients who had homozygous mutation of ribonuclease L (RNase L) especially the R462Q variant [6]. RNase L is an endoribonuclease that is important in the antiviral defence of the

FIG. 1. The three phases of immunoediting: Elimination-the process of identifying and eradicating tumour cells; Equilibrium- where the proliferation and destruction of the tumour cells is at a balance; Escape- where the tumour growth outweighs its elimination. NK cells are natural killer cells whilst NKT are variants expressing an extremely limited T cell repertoire; $CD4^+$, $CD8^+$, $CD4^+CD25^+$ Treg, and γδ cells are all types of T cell. (Figure published with permission of Enid Hajderi, University of Toronto, Division of Biomedical Communications).



BOX 1. T lymphocytes.

T cells are lymphocytes that play an important role in cell mediated immunity. They have T cell receptors on their surface and are categorised into the following subsets: T helper, cytotoxic, memory, regulatory and gamma delta T cells.

- *T helper cells (Th1, Th2, and others) express CD4 and support other lymphocytes in their immunological actions.*
- *Cytotoxic T cells (CTL) express CD8 and eradicate tumour cells and cells infected by viruses.*
- *Memory T cells are antigen specific T cells that are able to expand and mount a large immune response on re-exposure to an antigen.*
- *Regulatory T cells (Treg) inhibit other T cells in order to maintain immunological tolerance.*
- *Gamma delta T cells (γδT) have different types of T cell receptor subunits and are able to recognise molecules other than classical peptide/HLA complexes, such as glycolipids.*

host, suggesting that impaired immunity against this pathogen may have been associated with the risk of developing malignancy. Another study which looked at 334 CaP specimens found 23% of the samples to have XMRV protein expression in high grade tumour [5]. However in this study the XMRV infection and the development of cancer were independent of RNase L gene mutation. In contrast, a study conducted in Germany that examined more than 500 tissue samples showed no evidence of infection [7]. XRMV may thus exhibit variable geographic distribution. Its role in prostate carcinogenesis remains tantalising.

One of the implications of the association between CaP from XMRV is that the possibility for a preventative or therapeutic vaccine exists.

ROLE OF IMMUNITY IN CONTROL OF DISEASE (IMMUNOEDITING)

Inflammation may either promote or inhibit tumour growth, depending on context. In the 1950s, Burnet and Thomas put forward the 'immune surveillance' hypothesis, where the immune system has the ability to recognise and destroy nascent transformed cells [8]. However, this hypothesis subsequently fell into disrepute. The broader term 'cancer immunoediting', which was developed by Robert Schreiber and colleagues, was more appropriate to describe the host-protecting and tumour-sculpting actions of the immune system [9]. In cancer immunoediting, there are three proposed phases: (i) elimination; (ii) equilibrium; and (iii) escape (Fig. 1). These are commonly referred to as the three Es of cancer immunoediting:

ELIMINATION

The elimination phase encompasses the original cancer immune surveillance hypothesis in which there is successful eradication of the developing tumour. The process of elimination includes innate and adaptive immune responses to the tumour cells. Important components of the immune system are T lymphocytes and natural killer cells (summarised in Box 1).

There are four phases in the elimination process. The first phase is where cells of the innate immune system are recruited to the tumour site via pro-inflammatory signals. T cells and NK cells produce IFN-γ. In the second phase IFN-γ and other cytokines, chemokines and inflammatory molecules induce the production of chemokines from tumour cells as well as the surrounding normal tissue. These chemokines cause tumour death by anti-angiogenic effects and NK and macrophage recruitment. In the third phase, the recruited tumour-infiltrating NK and macrophages kill more tumour cells by activating cytotoxic mechanisms such as tumour necrosis factor-related apoptosis-inducing ligand, perforin and reactive oxygen and nitrogen intermediates. Within local lymph nodes, dendritic cells that have migrated from the inflamed region induce tumour-specific $CD4^+$ T helper cells, which in turn facilitate the development of tumour-specific $CD8^+$ T cells. During the fourth phase, the tumour-specific $CD4^+$ and $CD8^+$ T cells travel to the tumour site. Here the cytotoxic T lymphocytes eradicate the remaining

TABLE 1 Classification of tumour epitopes and targets. Please refer to http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm for the peptide database

Classification of epitopes/antigens	Description	Example
Unique tumour specific antigen	Mutation occurring in a single tumour of a patient	Point mutation found in melanoma-associated-mutated antigen-1 (MUM1)
Shared lineage-specific differentiation antigens	Can be expressed in patients with a tumour or in healthy individuals	Prostate antigens PSA and Kallikrein 4
Shared tumour-specific antigens	Expression predominantly restricted to cancer and not normal cells	'Cancer-testis' antigens such as MAGE family members and NY-ESO-1
Overexpressed antigens	The expression of these antigens are not tumour specific but a high level accounts for tumour presence	PSMA, DKK1, ENAH and STEAP1
Viral antigens	Xenoantigens due to oncogenic viruses	XMRV

tumour cells which have been exposed to local IFN-γ.

EQUILIBRIUM

The equilibrium stage is where the tumour cells that have survived the immune surveillance and the host immune system are in balance. There may be ongoing destruction of tumour cells by lymphocytes but this immune pressure in turn leads to selection and 'sculpting' of the antigenic profile of the tumour cells, leading to variant mutations able to resist the immune attack. This phase may be the longest of the three phases and is thought to often proceed over many years.

ESCAPE

Finally during the escape phase, the tumour cells have evaded detection and elimination by the host immune system either by genetic or epigenetic transformation. These cells proliferate uncontrollably resulting in dissemination of the tumour variant. If this process is unhindered, the outcome will be the demise of the host.

Mechanisms by which CaP can escape the host's immunogenic defences include defective antigen presentation, expression of immunosuppressive molecules, T cell receptor dysfunction, and active immune down-modulation through mechanisms such as T regulatory cells or myeloid-derived suppressor cells.

DEFECTIVE ANTIGEN PRESENTATION

T cells recognise antigenic peptides presented on HLA complexes [10]. Dendritic cells are antigen-presenting cells that lead to specific immune responses as they are able to activate antigen-specific T cells. Tumour cells can evade recognition and eradication from cytotoxic T lymphocytes if they have defective antigen presentation, such as down-regulation of HLA class I expression, defective antigen processing, or simply loss of antigen expression. HLA class I antigen expression has been shown often to be reduced in CaP. This implies that this mechanism of immune evasion is feasible in CaP, and that selective pressure has occurred in vivo in order to select for cancer clones with low levels of expression [11]. In CaP tissue, dendritic cells have been found to be reduced in number in comparison to normal prostate tissue [12]. They were also found to be minimally activated. However the mature dendritic cells in peripheral blood were found to be fully functional and able to be targeted with immunotherapeutic treatments [13].

IMMUNOSUPPRESSIVE SUBSTANCES

Immunosuppressive cytokines such as interleukins (IL) have been found to be present in higher levels in serum of CaP patients in comparison to normal controls. The presence of high levels of IL-6 has been shown to be a poor prognostic factor and also to have direct effects on tumour growth and T cell dysfunction [14]. IL-6 and its receptors are expressed in the epithelium and stroma of prostate tissue. As a result, cytokines may regulate CaP growth in an autocrine and paracrine manner.

Another immunosuppressive substance is transforming growth factor-beta (TGF-β). It is a cytokine that controls proliferation, and plays a role in many immune processes such as homing, cellular adhesion, chemotaxis and T cell activation, differentiation and apoptosis. It is also involved in CaP cell growth whereby stromal cells deficient in TGF-β responsiveness were prone to tumourigenesis via an oncogenic signalling pathway using Wnt3a proteins.

L-arginine is an amino acid; it is metabolised by nitric oxide synthase (NOS) to produce free radicals such as nitric oxide and L-ornithine. The increased metabolism of L-arginine within tumours has been suggested as resulting in tumour growth, angiogenesis, metastasis and tumour-related immunosuppression [15]. Furthermore, studies have shown that high levels of NOS is related to poor survival in CaP [16].

Indoleamine 2,3-dioxygenase (IDO) is another molecular mechanism that suppresses the immune system. Tumour induced tolerance is achieved by direct suppression of T cells and enhancement of Treg-mediated immunosuppression [17]. Other activators of antitumour immunity are also antagonised by IDO.

Finally, cyclooxygenase-2 (COX-2) is an enzyme that converts arachidonic acid to prostaglandins. It is often overexpressed and activated in inflammation and cancers [18]. In CaP, a high COX-2 expression has been associated with a high Gleason score, local chronic inflammation and tumour neovascularisation [19]. Immunosuppression and pro-tumourigenic effects such as inhibition of apoptosis, angiogenesis and

TABLE 2 Current Immunotherapy trials for Prostate Cancer. Please refer to http://www.clinicaltrials.gov for more details

Immunotherapy Agent	Name of trial	Trial Reference number	Trial Phase
Sipuleucel-T	A Randomized, Double Blind, Placebo Controlled Phase 3 Trial of Immunotherapy With Autologous Antigen Presenting Cells Loading With PA2024 (Provenge®, APC8015) in Men With Metastatic Androgen Independent Prostatic Adenocarcinoma	NCT00065442	III
Sipuleucel-T	A Single Center, Open Label, Phase 2 Trial of Immunotherapy With Sipuleucel-T as Neoadjuvant Treatment in Men With Localized Prostate Cancer	NCT00715104	II
Sipuleucel-T	An Open Label, Single Arm Trial of Immunotherapy With Autologous Antigen Presenting Cells Loaded With PA2024 (APC8015F) for Subjects With Objective Disease Progression and Disease-Related Pain on Protocol D9902 Part B	NCT00170066	II
Sipuleucel-T	A Randomized, Multicenter, Single Blind Study in Men With Metastatic Androgen Independent Prostate Cancer to Evaluate Sipuleucel-T Manufactured With Different Concentrations of PA2024 Antigen	NCT00715078	II
Sipuleucel-T	Immunotherapy For Men With Objective Disease Progression On Protocol D9902 Part B	NCT00849290	II
Sipuleucel-T	An Open Label Study of Sipuleucel-T in Men With Metastatic Castrate Resistant Prostate Cancer	NCT00901342	II
Sipuleucel-T	Autologous PAP-Loaded Dendritic Cell Vaccine (APC8015, Provenge [TM]) in Patients With Non-Metastatic Prostate Cancer Who Experience PSA Elevation Following Radical Prostatectomy: a Randomized, Controlled, Double-Blind Trial	NCT00779402	III
Autologous Natural Killer / Natural Killer T Cell Immunotherapy	A Phase I Open Label, Single Site, Safety and Efficacy Study of the Effects of Autologous Natural Killer and Natural Killer T Cell Immunotherapy on Malignant Disease	NCT00909558	I
NY-ESO-1/LAGE-1 HLA class I/II peptide vaccine	Immunotherapy of Patients With Androgen-Independent Prostate Carcinoma Using NY- ESO-1/LAGE1 Peptide Vaccine	NCT00711334	I
NY-ESO-1 class I and class II peptide vaccine LAGE-1 class I and class II peptide vaccine	Immunotherapy of Patients With Androgen-Independent Prostate Carcinoma Using NY- ESO-1/LAGE1 Peptide Vaccine	NCT00616291	I
Autologous dendritic cells transfected with amplified tumor RNA	A Safety and Feasibility Study of Active Immunotherapy in Patients With Metastatic Prostate Carcinoma Using Autologous Dendritic Cells Pulsed With Antigen Encoded in Amplified Autologous Tumour RNA	NCT00006430	I
Ipilimumab	A Randomized, Double-Blind, Phase 3 Trial Compßaring Ipilimumab vs. Placebo Following Radiotherapy in Subjects With Castration Resistant Prostate Cancer That Have Received Prior Treatment With Docetaxel	NCT00861614	III
IL-2 plasmid DNA/lipid complex	Phase II Study Evaluating the Safety and Efficacy of Immunotherapy Neoadjuvant Leuvectin for the Treatment of Prostate Cancer	NCT00004050	II
Prostatic acid phosphatase-sargramostim fusion protein + Sipuleucel-T	A Randomized, Double Blind, Placebo Controlled Trial of Immunotherapy With Autologous Antigen-Loaded Dendritic Cells (Provenge) for Asymptomatic Metastatic Hormone-Refractory Prostate Cancer	NCT00005947	III
IL-2 plasmid DNA/lipid complex	Phase I/II Study Evaluating the Safety and Efficacy of Leuvectin Immunotherapy for the Treatment of Locally Recurrent Prostate Cancer Following Radiation Therapy	NCT00005072	I & II
Peptide vaccine (PSMA and TARP peptide vaccine with Poly IC-LC adjuvant)	Pilot Immunotherapy Study of Combination PSMA and TARP Peptide With Poly IC-LC Adjuvant in HLA-A2 (+) Patients With Elevated PSA After Initial Definitive Treatment	NCT00694551	Pilot
ALECSAT	A Prospective, Open Phase I Study to Investigate the Tolerability and Efficacy of Administering ALECSAT to Prostate Cancer Patients – a First Dose in Man Study	NCT00891345	I
Adenovirus/PSA Vaccine	Phase II Study of Adenovirus/PSA Vaccine in Men With Hormone – Refractory Prostate Caner	NCT00583024	II
Adenovirus/PSA Vaccine	Phase II Study of Adenovirus/PSA Vaccine in Men With Recurrent Prostate Cancer After Local Therapy	NCT00583752	II
Autologous dendritic cell vaccine (DC/PC3)	A Phase I/II Study of Autologous Dendrtitic Cells Pulsed With Apoptotic Tumor Cells (DC/PC3) Administered Subcutaneously to Prostate Cancer Patients.	NCT00345293	I & II
PSMA/PRAME	A Phase I Multicenter, Open Label, Clinical Trial of Immune Response, Safety and Tolerability of DNA Vector pPRA-PSM With Synthetic Peptides E-PRA and E-PSM in Subjects With Advance Solid Malignancies	NCT00423254	I

enhanced cell invasiveness have all been linked with COX-2 actions.

T CELL RECEPTOR DYSFUNCTION

Antigens are recognised by T cells via the T cell receptor (TCR) complex. Diminished activation of TCR has been implicated in many tumours and may be a mechanism of tumour escape. Also co-inhibitory signals such as CTLA-4, PD-1, SHP1 can inhibit TCR signalling and down-regulate the immune activity [20].

ACTIVE IMMUNE DOWN-MODULATION

T regulatory cells are a subset of T cells that express CD25, the ∝–chain of the interleukin 2 (IL-2) receptor and FOXP3, a transcription factor. They play a role in ongoing active suppression of immune responses to normal self-antigens, thereby protecting against clinical autoimmunity such as inflammatory bowel diseases [21]. There is evidence of a high frequency of T regulatory cells in CaP tissue allowing for the tumour cells to escape the inhibitory effects of the immune system [22].

Myeloid-derived suppressor cells (MDSC), have suppressive properties in cancer where they inhibit the activation and proliferation of tumour specific T cells and also induce apoptosis. They originate from haematopoietic progenitor cells and are released into circulation to localise in the tumour microenvironment and lymph nodes. Their mode of action is by secretion of factors such as reactive oxygen species, NO, IL-10 and TGF-β [23].

USE OF IMMUNE STRATEGIES TO TREAT CANCER

Despite the escape mechanisms that alter host immunity, most CaP patients are immunocompetent. The inability of the immune system to act on the tumour represents immunological tolerance. However with the advances in identifying new immune targets and T cell epitopes, tumour immune targeting has improved (Table 1).

The role of anti-tumour active immunotherapy is to generate a T cell response to recognise and destroy tumour cells [24]. Active immunotherapy functions by inducing T cells against a specific tumour antigen and it can be delivered in the form of a vaccine. There are numerous immunotherapy trials in progress (Table 2).

One approach tested recently in castrate resistant CaP is the GVAX vaccine. This preparation comprises cells from CaP cell lines, LNCaP and PC3, that are genetically modified to produce granulocyte macrophage-colony stimulating factor (GM-CSF). The GVAX vaccine is recognised as a source of foreign antigens and antigen presentation is enhanced by the presence of the GM-CSF. Antigen-loaded APC travel to lymph nodes and stimulate and activate $CD4^+$ and $CD8^+$ T cells resulting in an immune reaction against the antigen. The initial phase I and II studies of GVAX were promising however phase III studies named VITAL-1 and VITAL-2 had to be terminated due to a higher death rate in the GVAX arm in VITAL-2 and futility analysis of VITAL-1 showing less than 30% chance of obtaining a survival benefit. As a result the future use of this vaccine is not clear.

In contrast, the most recent immunotherapeutic agent showing promise in CaP is Sipuleucel-T, also known as APC8015 or Provenge® (Dendreon). This treatment involves central processing and preparation of the therapeutic product, comprising autologous antigen presenting cells (APC) primed with a fusion protein of prostatic acid phosphatase (PAP), a molecule that is commonly expressed on CaP cells. The PAP antigen is linked to GM-CSF, an immune cell activator. Activated and antigen-loaded APCs present these antigens to T cells and stimulate and expand effector and memory T cells. In patients with CaP, activation of immature dendritic cells leads to DC maturation and their mobilisation to regional lymph nodes, resulting in more efficient antigen processing and presentation [25]. The IMPACT trial, a multi-centre, randomised, double blinded, placebo-controlled study treated men with asymptomatic metastatic androgen-independent CaP with the immunotherapeutic agent Sipuleucel-T [26]. When compared to the placebo, Sipuleucel-T improved median survival by 4.1 months, improved three-year survival by 38% and reduced the overall risk of death by 22.5% while having a modest toxicity profile [27]. Sipuleucel-T has now been approved by the United States Food and Drug Administration for this indication.

The combination of chemotherapy and immunotherapy has been shown to be advantageous. A recent paper looking at the use of cyclophosphamide and dendritic cell based immunotherapy in men with hormone refractory metastatic CaP showed that the treatment led to improvement in clinical status and also measures of tumour burden such as PSA and ALP [28]. The cyclophosphamide was initially used to reduce the number of circulating Tregs before administering the DC vaccine. Such combination approaches may prove to be an effective form of management.

CONCLUSION

In CaP, an understanding of the mechanisms of inflammation and the possible routes via which tumours evade the scrutiny of the immune system offers a promising route to enhance survival and reduce progression, particularly in combination with other therapies. Recent and current immunotherapy trials suggest that improved outcomes might be achievable. Further research is still required into understanding the immune response so that the use of multiple therapies can be best employed to synergistically improve therapeutic effects.

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CONFLICT OF INTEREST

None declared.

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