A primer on tumour immunology and prostate cancer immunotherapy

Runhan Ren;[1] Madhuri Koti, DVM, MVSc, PhD;[2] Thomas Hamilton, DVM, MVSc, PhD;[2] Charles H. Graham, PhD;[1,2] Jasmir G. Nayak, MD;[3] Jas Singh, MD;[3] Darrel E. Drachenberg, MD;[3] D. Robert Siemens, MD[1,2]

[1]Department of Urology and [2]Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada; [3]Section of Urology, University of Manitoba, Winnipeg, MB, Canada

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Introduction

Prostate cancer is the most commonly diagnosed non-cutaneous malignancy and the third leading cause of cancer death in Canadian men.[1] Despite this incidence and the significant health burden associated with prostate cancer, its management over the last decade has become increasingly complex and controversial for both early and advanced disease. Recent recommendations questioning the benefit of prostate-specific antigen (PSA) screening[2] have highlighted the requirement to uncouple the diagnosis from treatment for localized disease in order to reduce over-treatment of biologically indolent disease.[3] In the meanwhile, trends to decreased screening in an aging population could result in significant increases in men with more advanced disease requiring salvage or palliative treatment, including androgen-deprivation therapy (ADT) and systemic chemotherapy.

Despite recent interest in the earlier delivery of cytotoxic chemotherapeutics for men with advanced disease, prostate cancer remains, for the most part, androgen-dependent. In a non-curative setting, ADT remains a mainstay of treatment. Although most patients are initially responsive, progression to castration-resistant prostate cancer (CRPC) eventually occurs and is associated with a median time until death of less than three years.[4] Nevertheless, this past decade has seen substantial improvements in the management of CRPC.[5] Docetaxel, approved by Health Canada in 2005, was the first drug to demonstrate survival benefit for men with metastatic CRPC. An increasing understanding of the mechanisms of survival in prostate cancer cells with castrate levels of serum testosterone has led to multiple new therapies, including bone-targeted agents and next-generation androgen receptor inhibitors.[5] Overall, these new therapeutic modalities have led to improvements in the quantity and quality of life of men with CRPC.

Unfortunately, progression to a chemo-resistant, androgen-independent state is the norm. Exploring other therapeutics, including those processes and pathways involved in resistance to standard therapies, is key to further improving the quality and quantity of life of these patients. Immunotherapy represents one potentially innovative and complementary management strategy for those with advanced prostate cancers. In April 2010, the FDA approved Sipuleucel-T vaccine for the treatment of metastatic CRPC patients, making it the first therapeutic vaccine for any cancer.[6] Less than a year later, the immune-stimulating drug ipilimumab was approved for the treatment of metastatic melanoma, ushering in even more focus on the potential of cancer immunotherapy.[7]

Recent advances in our understanding of immune interactions with cancer cells, leading to these and other successful therapeutic strategies to harness the power of the patient's own immune system, mark the beginning of an exciting new era in cancer management. For urologists, medical oncologists, and other clinicians that regularly care for men with prostate cancer, remaining up-to-date with these new therapies and their underlying immunological concepts will allow them to offer, and better explain, the most appropriate therapies for their patients. Here we review the basic concepts in tumour immunology that underlie cancer immunotherapy with a primary focus on prostate cancer immunotherapies.

Tumour immunology: Hallmarks of anti-tumour immune responses

A few key concepts are worth reviewing with respect to what is known about immune detection and elimination of tumour cells. The notion that the immune system acts as an extrinsic tumour suppressor by preventing the proliferation of neoplastic cells was first proposed by Ehrlich in the early 20th century.[8] It is now well established that transformation to a malignant cell involves production of cell surface markers, also called tumour-associated antigens (TAAs), which

are recognized by the immune system as non-self, and thus are the initiating steps in an anti-tumour immune response.[9]

Since Ehrlich's time, data gathered from various studies have provided evidence supporting the idea that the immune system plays an important role in both cancer progression and suppression, a concept now referred to as immune surveillance.[10] Cancer immunotherapies exploit these concepts and thus aim at strengthening tumour immune surveillance. In order to better understand immunotherapy, we must first understand normal immune system function and its role in tumour cell killing/evasion. Both divisions of the immune system, *the innate*[11] and *adaptive,*[12] have been shown to be involved in tumour immune surveillance and thus have been targets across immunotherapies.

Anti-tumour *innate* immune responses

Innate immune cells are responsible for the initial immediate response to tissue damage and play a role in preventing and facilitating tumour progression. Macrophages are initially recruited and can be classified as pro-inflammatory M1 cells and anti-inflammatory M2 cells, with a functional spectrum existing between the two ends.[13] Under the influence of tumour-derived or environment-derived soluble mediators, such as cytokines, the relationship between M1 and M2 cells can become unbalanced.[14] Tumours can develop immunity to the initial pro-inflammatory attack and differentiate the macrophages into tumour-promoting cells, called tumour-associated macrophages.[11] In many ways, the tumour-associated macrophages, similar to M2 cells, are anti-inflammatory and involved in promoting a favourable tumour microenvironment. One potential therapeutic target for tumour-associated macrophages is the receptor for colony-stimulating factor 1, a vital growth factor for macrophages and their differentiation, migration, and survival.[15]

Natural killer cells are a distinct population of lymphocytes capable of targeting tumour cells without prior sensitization. They recognize foreign and altered cells by two mechanisms: missing-self and altered-self.[16] Missing-self refers to the ability of natural killer cells to detect the loss of major histocompatibility complex (MHC) class I molecules — self-surface cell makers — a change that is associated with damaged, transformed, and/or infected cells. In contrast, the altered-self mechanism refers to the process by which damaged cells express specific ligands — TAAs— that trigger natural killer cell activity.[16]

Antigen-presenting cells (APC), such as macrophages and dendritic cells (DCs), are important in anti-cancer immunity by being the link between the innate and adaptive immunity. DCs have many receptors that recognize specific pathogen-associated molecular patterns and environmental signals. Upon activation, they migrate to sentinel lymph nodes and present antigens and activate naïve T cells.

Anti-tumour *adaptive* immune responses

The adaptive immune system is constituted by T and B lymphocytes that mediate specific functions via cell surface or secreted effector molecules. The process starts when a naïve helper T cell encounters an APC with a MHC II-bound antigen complementary to its own T cell receptor. Adaptive immunity has long been recognized in the eradication of tumour cells.[17]

Cell-mediated immunity

The main effector cells in cancer immune response are CD8+ cytotoxic T-lymphocytes (CTL). The antigens that trigger these responses are often peptide fragments of TAAs from the initial tumour cell destruction by innate effectors, such as natural killer cells, cytotoxic chemotherapy or radiation therapy, or tumour cell lysis by oncolytic viruses. Once activated, CTLs undergo clonal expansion, resulting in a population of cytotoxic cells specific to that tumour antigen.

CD4+ T cells, also known as helper T cells, induce antibody production in B cells, activate macrophages, and can recruit and regulate other immune effectors through the cytokines and chemokines that they secrete.[18] There are four subsets of CD4+ T cells: Th1, Th2, Th17, and regulatory T cells (Tregs) based upon their function and the cytokines they secrete. In general, Th1 cells are involved in intracellular immunity and Th2 in extracellular (humoral) immunity. Th17 cells are unique in their expression of IL-17. Tregs are an important subset of CD4 T cells that are able to suppress effector T cells and also maintain immune tolerance.[19] Tregs can be found in large proportions of tumour infiltrating lymphocytes, in comparison with other effector T cells, which have been associated with poor prognosis of certain cancers.[20] Considering the fact that most TAAs are normal self-antigens, Tregs may suppress normal anti-tumour immunity and be an obstacle for cancer immunotherapy. Tregs constitutively express CTLA-4 and targeting this important regulator to decrease Tregs in tumours has resulted in significant clinical benefit.[21] However, Tregs consist of heterogeneous subgroups and, while modifying their activity may lead to anti-tumour immunity, a negative outcome may include autoimmunity.[22]

Natural killer T (NKT) cells are immune cells that bridge the gap between innate and adaptive immunity. Type I NKT cells play a protective role in tumour immunity, they activate NK and CTLs and stimulate DCs to produce IL-12.[23] In contrast, type II NKT cells inhibit tumour immunity and counter-regulate type I cells.

Humoral immunity

The humoral arm, mediating its functions primarily by secreting antibodies and cytokines, is now gaining attention in the field

of tumour immunology, with its anti- and pro-tumourigenic roles across cancers.[24] For prostate cancer, recent data have suggested that B cell response to tissue damage after androgen depletion drives restorative or aberrant prostate proliferation.[25] Increased B cell infiltration in the prostate has been associated with malignant rather than benign tissue.[26] In addition, a small case series treating $CD20^+$ prostate cancer with anti-CD20 antibodies resulted in a significant biochemical response.[27]

Tumour immune escape

The body is normally able to suppress tumour cells through immune surveillance, which might help explain why most people never develop cancer. However, as part of tumour immuno-editing, tumour cells often gain properties to escape detection and establish themselves and present as disease.[8] Three phases have been proposed to explain this transition: elimination, equilibrium, and escape[28] also known as the three "Es" of cancer immuno-editing. Elimination of tumour cells occurs by innate and adaptive immune mechanisms. Pro-inflammatory cytokines, released by the growing tumour, macrophages, and surrounding stromal cells, activate and recruit several immune effectors. NK-mediated tumour destruction releases tumour associated antigens, which induce adaptive immune responses.

Elimination via CTL activation can result in the selection of tumour cells with reduced immunogenicity and thus become resistant to immune effectors. This results in tumour growth favouring non-immunogenic phenotypes.[29] During the equilibrium phase, elimination of tumour cells is balanced by the selection of less immunogenic variants.[30] As tumour size increases, tumour-derived soluble factors can cause several mechanisms of immune escape.[29] The soluble factors help to modify the tumour microenvironment, including the attraction of effector cells, such as immature DCs and fibroblasts. Some of these factors can lead to increased extracellular matrix that binds tumour antigens; fibroblasts and endothelial cells compete with DCs for antigens, effectively reducing the amount of TAAs and contributing to tumour progression.[31] Immature DCs also inhibit T cell activation and stimulate regulatory T cells, contributing to immune tolerance.[32] Immune escape can be, at least partly, attributed to these microenvironmental modifications of the decreased antigen levels fortified by reduced T cell activation.

The promise of immunotherapies in prostate cancer

Consequent to the tumour immunosurveillance theory, the past decade has witnessed significant successes in cancer immunotherapies that rely on enhancing the effectiveness of host anti-tumour immune responses in multiple ways.[6] Most significant improved survival rates have been achieved with the use of immunotherapies targeting the immune checkpoint regulators PD-1/PD-L1 and CTLA-4,[33] which recently showed success in melanoma and lung cancer trials.[34] Recent research has shown emphasis towards combinatorial therapies for increased clinical benefit.[35]

Alterations in the tumour cells lead to constantly evolving tumour mutanome/antigenome that allows activated T cells and their effectors to infiltrate the tumour microenvironment.[28] In prostate cancer, many of the tumour-associated antigens, such as PSA, prostatic acid phosphatase (PAP), and prostate-specific membrane antigen (PSMA), lead to a T cell response. Prostate cancer is often a slow proliferative disease, causing many cytotoxic agents to be ineffective. However, it provides the time needed to mount an immune response, even in patients with advanced or metastatic disease.[6]

Prostate cancer vaccines

Passive immunotherapy

Immunotherapy is based on agents capable of enhancing anti-tumour immunity by either bolstering the patient's own immune effectors or by passively providing immune effectors that are already primed to kill the patient's tumour. Passive immunotherapy uses anti-tumour agents generated *in vitro*. Monoclonal antibodies can be generated against specific tumour surface markers. For prostate cancer, these agents are still early in development and have mainly focussed on PSMA.[36] As a TAA, PSMA is overexpressed on the tumour cell surface and associated vasculature.[37] Clinical trials have shown good tumour targeting, but few objective clinical responses, and more recent studies have focused on radiolabelling for radiotherapy.[38]

Building on the idea of passive vaccination, adoptive cell therapy is a form of personalized medicine involving the transfer of activated autologous or allogenic T cells into the patient.[39] The goal is that the modified T cells are able to stimulate anti-tumour responses, increase vaccine efficacy, and limit graft-vs-host disease. The T cells are engineered with surface receptors that recognize specific TAAs, termed chimeric antigen receptors. This essentially bypasses the steps of tumour recognition, T cell activation, and amplification required in the body. Several clinical trials are testing transfer of tumour-infiltrating lymphocytes, CTLs, Th cells, and Tregs.[39] Early-stage trials have shown success in advanced leukemia and lymphoma.[40]

Active immunotherapy

In contrast to passive immunotherapy, active immunotherapies aim to generate an active immune response against TAAs. Immunotherapeutic vaccines can be separated into four classes: autologous, cell, DNA, and viral-vector based.

Autologous vaccines

Sipuleucel-T is an autologous vaccine in which the patient's own peripheral blood mononuclear cells, along with APCs, are retrieved via leukapheresis. The cells are then activated with a recombinant fusion protein consisting of PAP linked to granulocyte-macrophage colony stimulating factor (GM-CSF), an immune cell activator.[41] Sipuleucel-T was approved by the FDA in 2010, after a large, multicentre phase III trial (IMPACT), resulting in a 4.1-month overall survival benefit and a 22% relative risk reduction of mortality in patients with metastatic CRPC.[41] The treatment was well-tolerated, with minimal adverse events. However, there remain several unanswered questions that have limited its uptake in North America. The treatment is currently cost-prohibitive, with a cost-utility ratio of $283,000 per quality-adjusted life-year, and with limited availability.[42] Furthermore, several authors have questioned the phase III findings, specifically around the rationale of the placebo groups, a possible age bias, as well as the fact that no objective responses (declining PSA) were seen in the trials.[43]

Cell-based vaccines

GVAX is an allogenic cell-based prostate cancer vaccine that is composed of both hormone-sensitive and -naïve prostate cancer cell lines that have been genetically modified to bear GM-CSF.[44] GM-CSF results in recruitment of DCs, which then present antigens to T-cells invoking an anti-tumour cascade of immune responses. The whole tumour cell is used as the antigen, rather than just the PAP, as in sipuleucel-T, facilitating both humoural and cellular immune responses. Initial phase I/II studies confirmed safety, clinical activity, and immunogenicity;[44,45] however, two phase III studies (VITAL-1, VITAL-2)[46] were closed early due to futility analysis.

DNA-based vaccines

DNA-based vaccines consist of bacterial plasmids constructed to contain the coding sequence of a targeted antigen, which can be taken up by cells. These transformed cells express genes that can induce an immune response. Bacterial plasmids are attractive in their simplicity, stability, and cost-effectiveness, which can be encoded with adjuvants and cytokines to increase their immune response.[47] Phase I trials have been done targeting various TAAs, including PSA, PSMA, PAP, and the cancer-testis antigen NY-ESO-1.[48] These early trials have demonstrated vaccine safety, but poor immunogenicity, possibly due to poor transfection rates of APC.[48] Ongoing studies hope to address the preferred target antigen, administration, and disease stage.

Viral-based vaccines

Prostvac-VF is a vaccine comprised of two recombinant viral vectors that each encode for PSA and three immune co-stimulatory molecules including: co-stimulatory molecule for T cells (B7-1); intracellular adhesion molecule 1, and lymphocyte function-associated antigen 3.[49] The vaccinia virus-based vector is used for priming and is followed by fowl pox virus-based vector boosts. This helps to overcome the host anti-vector antibody responses to the original vector. GM-CSF is co-administered to further boost immune response. The virus infects APCs, promoting cell surface protein expression and interaction with T-cells that facilitate a targeted immune response and cell-mediated tumour cell destruction.[50] Current trials are testing Prostvac-VF in men with castration-resistant disease, as well as in combination with other traditional therapies and immunotherapies.[6]

Immune checkpoint blockade as an emerging pillar in the treatment of prostate cancer

Immune responses are kept in balance by immune checkpoints that oppose co-stimulatory pathways, as well as clonal selection, activation, proliferation, trafficking, and effector function. Under normal conditions, these mechanisms help maintain self-tolerance, duration, and strength of immune responses, and aim to minimize damage to surrounding self-tissues.[34] Alteration of these pathways in tumour cells re-directs T cell-mediated immunity such that checkpoint regulating molecules altered within the tumour or surrounding immune cells send a negative signal into the binding T cell, thus leading to its exhaustion. Recent successful immunotherapies have extensively exploited these mechanisms to enhance immune-mediated tumour cell destruction.

CTLA-4 based immunotherapy

T cell activation initiates several downstream functions, such as cytokine production, cell cycle progression, and effector differentiation. The B7 family of cell-surface ligands are found on APC and T cells, which bind to CD28 receptors on lymphocytes.[51] Activation of T cells requires two signals: antigens presented on HLA receptor of APC and B7 co-stimulatory molecule of APC and its CD28 receptor on T cells. T cell activation induces expression of inhibitory signals, which limit and control the immune response. CTLA-4 is a co-inhibitory signal that binds B7 with greater affinity. CTLA-4 blockade removes the inhibition and results in T cell activation against tumour cells.[52] Ipilimumab is a human monoclonal antibody against CTLA-4, first approved for metastatic melanoma in 2011. Several current trials are testing ipilimumab in patients with prostate cancer as a monotherapy and in combination settings. To date, monotherapy with ipilimumab in a phase III trial assessing men with castration-resistant disease was negative overall; however, there has been demonstration of good biochemical

response and there was a signal of a survival benefit in subgroups of patients with favourable prognostic features.[34]

PDL-1/PD-1 axis-based immunotherapy

Programmed cell death (PD) 1 is a cell surface molecule on T-cells that interacts with ligands, including PD-L1. This interaction inhibits downstream T cell receptor signalling, preventing T cell activation leading to their exhaustion and subsequent apoptosis.[53] PD-L1 is expressed on a variety of cells, such as T, epithelial, endothelial, and tumour cells after exposure to IFN-γ.[54] This interaction helps to regulate the immune response by reducing autoimmunity and developing self-tolerance. Similar to CTLA-4 targeting by ipilimumab, PD-1 is an additional but non-redundant pathway for which inhibition results in a targeted anti-tumour response. There has been recent success with anti-PD-1 therapy in advanced melanoma, with the FDA approval of pembrolizumab in September 2014, and nivolumab in December 2014 for metastatic melanoma.[35] In March 2015, nivolumab was approved for advanced or metastaic non-small-cell lung cancer. Currently, pembrolizumab is being investigated in a phase II trial in metastatic CRPC after ADT (NCT02312557). Pidlizumab is another PD-1 monoclonal antibody currently being investigated in metastatic CRPC in combination with Sipuleucel-T and cyclophosphamide (NCT01420965).

These two checkpoint regulators have different, non-overlapping mechanisms of action and can thus ideally be used in combination to maximize immune response. In theory, anti-CTLA-4 therapy increases infiltrating T cells and IFN-γ, which induces PD-L1 expression. This would increase the response from PD-1 targeting. Results of a phase III study evaluating combination treatment of ipilimumab and nivolumab vs. monotherapy in patients with unresectable stage III or IV melanoma were recently published.[55] The median progression-free survival was significantly higher in combination treatment; however, so were treatment-related adverse events. New emerging immune checkpoint targets have been identified and include LAG-3, TIM-3, VISTA, and co-stimulatory molecules OX40, ICOS, and 4-1BB.[35]

Conclusions and future directions

The promise of cancer immunotherapy appears to be on the verge of delivering. Most of us in the urological community have likely always been believers to some degree, given modest past successes in renal and bladder cancer. However, this has been a long time coming since the first observations in the early twentieth century by Coley using heat-killed bacterial infections to initiate an anti-tumour response.[56] Today, we see an increasing number of therapies focused on harnessing the anti-tumour T cell immune response, with many providing significant clinical benefit across various cancers. This progress has been a result of significant advances in our understanding of the complex nature of the regulatory events in cytotoxic T cell-mediated immune responses, particularly antigen presentation, activation and immuno-editing in the cancer microenvironment.

We hope this review serves as a primer of the intricacies of the immune system and cancer immunotherapy, as well as highlights some of the promising novel immunotherapeutic approaches being investigated in prostate cancer. Undoubtedly, these are early days and despite some encouraging and long-lasting responses in some heavily pre-treated patients with prostate cancer, there remains a great deal of both experimental and clinical investigation. Current approaches targeting only a discreet number of TAAs are destined to be limited; exploring other promising targets, such as ICOS, LAG3, VISTA, and OX40, may be required to extend the therapeutic benefits of current immunotherapies. There is substantial evidence suggesting that linking different immunotherapeutic approaches, as well as combining other local or systemic cancer therapies, will likely be required to realize synergistic benefits. This could be a daunting task, given the non-classic cancer responses to immunotherapy and co-evolving immune escape mechanisms, the need for knowledge-based trials to help inform dosing, timing, and sequencing, as well as the need to develop precise criteria for patient selection. Finally, as these novel therapies become more available, those in the urological community will need to become better educated regarding the recognition and management of immune-related adverse effects in order to maximize clinical benefit.

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Correspondence: Dr. D. Robert Siemens, Kingston General Hospital, Kingston, ON, Canada; siemensr@kgh.kari.net