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SYMPOSIUM PAPER

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Tumor escape mechanisms in prostate cancer

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Abstract Numerous immunotherapy trials have been carried out in prostate cancer (PC) patients, with induction of antigen-specific T cells in some cases. Despite this capability, limited success is seen in terms of tumor regression or survival. In this review, we discuss the evidence for tumor escape strategies that may contribute to vaccine failure in the setting of PC. These include defects in antigen presentation, production of immunosuppressive substances, induction of T cell death, T cell receptor dysfunction, and the presence of tolerogenic dendritic cells and regulatory T cells inside prostate tumors. It is clear that novel strategies aimed at preventing tumor escape, such as small molecular weight inhibitors of immunosuppressive molecules, adoptive transfer of TCR transgenic T cells, removal of Tregs, combined with anti-androgen therapy and prostate-specific vaccines, need to be examined further in PC patients.

Keywords Prostate cancer · Immunotherapy · T cell · Immune · Escape · Tumor

Introduction

Prostate cancer (PC) is currently the most commonly diagnosed cancer in men in Europe and the USA [1, 2]. Although the majority of cases are localized to the prostate, nearly one-third of newly diagnosed patients have advanced or metastatic PC. Radiation therapy and surgical resection can be curative in localized disease. However, no curative treatment currently exists when the disease spreads beyond the prostate. The identification of prostate tumor antigens that are recognized by T cells has created the opportunity to develop novel immunotherapeutic approaches, including tumor vaccines. Several antigens have already been identified, including prostate-specific antigen (PSA), prostatic acid phosphatase (PAP), and prostate-specific membrane antigen (PSMA) [3]. This led to numerous immunotherapy trials, including peptide [4], DNA [5], virus-based [6], dendritic cell [7], and genetically-modified tumor cell vaccines [8], in which induction of tumor-specific T cells could be shown in some patients [9]. However, in most cases limited success has been seen in terms of tumor regression and survival.

Vaccine failure may be attributed to several potential tumor escape mechanisms such as defects in antigen presentation, production of immunosuppressive substances, T cell dysfunction, and the presence of regulatory T cells [10–12]. In the following sections the evidence for infiltration of prostate tumors by T cells and tumor escape mechanisms in PC are discussed.

Do T cells infiltrate prostate tumors?

Many studies have shown the presence of $CD3^+$ T cells, including both $CD4^+$ and $CD8^+$ subtypes, inside prostate tumors. However, it is difficult to study the function of these cells for a number of reasons: prostate tumors are relatively small; surgery is only routinely carried out in early stage disease; the isolation of true tumor infiltrating lymphocytes (TILs) is problematic due to the infiltrative growth of PC within the prostate gland; low numbers of TILs are seen in PC [13]; the development of malignant ascites in PC is an extremely rare event with only a few published clinical case reports [14, 15]; and the generation of PC cell lines has proven difficult. Furthermore, the relatively few studies that have thus far looked at the relationship between lym-

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phocytic infiltration and survival in PC have shown conflicting data. Vesalainen et al. [16] reported that tumors with a dense tumor lymphocyte infiltration were associated with higher survival rates than tumors with absent or decreased infiltrates. In contrast, Irani et al. [17] reported that an increased inflammatory cell infiltrate within the tumor was associated with an increased risk of tumor recurrence. More recently, McArdle et al. [18] reported that the presence of $CD4^+$ T cell infiltrate was associated with poor cancer survival in patients with PC. One interpretation may be that patients who lack intratumoral T cells fail to mount an immune response to the tumor, while patients with intratumoral T cells are in the process of mounting an immune response, the success of which depends on the presence or absence of various tumor immune escape pathways.

Defects in antigen presentation

HLA class I antigens are critical for the recognition and lysis of tumor cells by cytotoxic T cells (CTLs), and defects in antigen presentation could allow the tumor to escape killing by CTLs (Fig. 1). Expression of HLA molecules and associated proteins required for efficient antigen processing, such as transporter associated with antigen processing (TAP), low molecular weight protein of the proteasome complex (LMP), and beta 2–microglobulin, were investigated in several human PC cell lines. HLA class I was under-expressed in two (LNCaP and PPC-1) PC cell lines [19, 20]. Furthermore, PPC-1 cells also under-expressed TAP-2 mRNA despite abundant HLA class I and beta 2-microglobulin message, and LNCaP cells had competent antigen transport but deficient HLA class I heavy-chain function despite abundant HLA class I RNA [19]. Increased shedding of beta 2-microglobulin was demonstrated in the PC-3 cell line, and increased levels of beta 2-microglobulin in the urine of PC patients was associated with a significantly shortened survival [21]. In contrast, no defects in the expression of class I antigen processing machinery (TAP-1, TAP-2, LMP-2, and LMP-7) were detected in a mouse PC cell line [22]. In primary human tissue, several studies report reduced or complete loss of HLA class I in prostate tumors and lymph node metastases, compared to the normal expression in benign tissue [20, 23–25]. However, other mechanisms for defects in antigen presentation, such as changes in the expression of TAP or LMP molecules, have not yet been reported in primary tumor tissue.

Production of immunosuppressive substances

Cytokines

It is postulated that an imbalance in Th1/Th2 cytokine production may be responsible for the development of cancer, with a shift toward a Th2 response and induction of immunosuppressive cytokines such as interleukin (IL)-4, IL-6, and IL-10 [26].

In the serum of PC patients elevated levels of several Th2 cytokines, such as IL-4, IL-6, and IL-10, were detected when compared to either patients with benign prostate hyperplasia or normal healthy controls [27–29]. Multiple studies have shown a role for IL-6 in PC progression and that increased serum levels of this cytokine are a significant indicator of poor prognosis in PC [30–32]. In prostate tumors it is postulated that IL-6 has a direct effect on tumor cell growth [33], and may also contribute to peripheral T cell dysfunction [11].

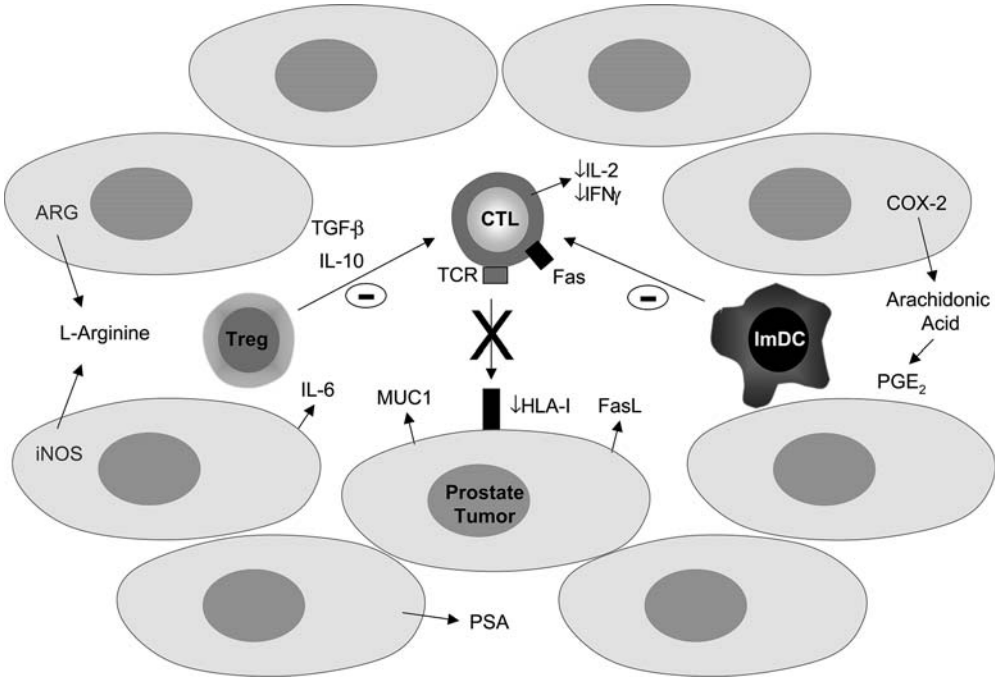

Fig. 1 A schematic representation of the potential mechanisms of immune escape in prostate cancer. Abbreviations: ARG arginase, COX-2 cyclooxygenase-2, CTL cytotoxic T cell, FasL Fas ligand, iNOS inducible nitric oxide synthase, IFNγ interferon-gamma, imDC immature dendritic cell, IL interleukin, MUC1 epithelial mucin 1, PGE_2 prostaglandin E_2, PSA prostate specific antigen, TCR T cell receptor, TGF-β transforming growth factor-beta, Treg regulatory T cell

Basal expression of cytokines, measured in T cells from blood of PC patients, demonstrated a lower expression of both IL-10 and IFNγ [29]. In cultured peripheral blood T cells from PC patients stimulated with PMA and/or ionomycin an increase in IL-10 and a decrease in IL-2, compared to healthy controls was reported in one study [27], while higher ratios of IFNγ/IL-4, IFNγ/IL-10, and IL-2/IL-4, a favorable type-1 cytokine pattern, were observed in patients with lower serum PSA compared to patients with high serum PSA [34]. Furthermore, direct study of prostate TILs showed a significantly higher expression of IL-10, compared to matched blood T cells [35]. In addition, TILs isolated from a rare malignant ascites in a PC patient were tumor antigen reactive but predominantly secreted Th2 cytokines upon stimulation [15].

Transforming growth factor-beta (TGF-β) is a cytokine that suppresses the immune system, and also has a complex role in the regulation of PC cell growth [22]. TGF-β can modulate many immune processes including homing, cellular adhesion, chemotaxis and T cell activation, differentiation and apoptosis. Recent progress in this field also suggests a key role for TGF-β in regulatory T (Treg) cell-mediated suppression. Several studies report upregulation of TGF-β expression in PC patients, both in the serum and in the tumors, and an inverse correlation with survival [36–38]. Although prostate tumor cells may lose sensitivity to this cytokine, via downregulation of the receptors, TGF-β may still be able to function as a potent immunosuppressive agent against incoming immune cells.

Amino acid metabolites

The amino acid L-arginine can be metabolized by the enzyme nitric oxide synthase (NOS), to generate the free radical nitric oxide (NO) and L-citrulline, or by the enzyme arginase (ARG), to generate urea and L-ornithine. In the prostate NO is reported to have a physiological role in smooth muscle tone and secretory functions [39, 40]. However, several studies in PC show that levels of the inducible form of NOS (iNOS) and ARG are increased inside the tumor [41–45], and that strong iNOS expression was a predictor for poor survival [46]. It is reported that increased L-arginine metabolism within tumors may contribute to tumor growth, angiogenesis, metastasis, and tumor-related immunosuppression [47, 48]. Recently, treatment of prostate tumors in culture with inhibitors of NOS (l-NMMA) or ARG (NOHA) resulted in upregulation of early activation markers on $CD8^+$ TILs, prolonged their survival and restored their lytic capacity [44]. It is therefore possible that defective L-arginine metabolism is one mechanism by which T cells are immunosuppressed inside prostate tumors.

In addition, expression of the tryptophan degrading enzyme, indoleamine 2,3-dioxygenase (IDO) in dendritic cells, was shown to have profound effects on T cell proliferation, differentiation, effector functions, and viability [49]. Thus far, there are no studies of this enzyme or its expression in PC models or patients and research into this area is warranted.

Arachidonic acid metabolites

Cyclooxygenase-2 (COX-2) is an enzyme that converts arachidonic acid to various prostaglandins and other eicosanoids. It is induced under various conditions, such as inflammation and cancer. COX-2 expression has been studied extensively in PC [50] and found in smooth muscle cells of both the normal and cancerous prostate. Luminal epithelial cells surrounded by lymphocytes are induced to express the enzyme in prostatic inflammation, and COX-2 is expressed in the epithelial cells of high-grade prostatic intraepithelial neoplasia and cancer. Recently, COX-2 expression was found to correlate with a higher Gleason score, local chronic inflammation, and tumor neovascularization in human PC [51]. COX-2 is suggested to play a key role in tumorigenesis through stimulating epithelial cell proliferation, inhibiting apoptosis, stimulating angiogenesis, enhancing cell invasiveness, and importantly mediating immune suppression. Prostaglandin E_2 (PGE_2), a product of arachidonic acid metabolism by COX-2, may regulate immune function by acting as a negative feedback inhibitor for various processes, including T cell proliferation, lymphokine production and macrophage and natural-killer cell cytotoxicity [52]. Therefore, inhibition of COX activity in PC may be associated with an enhanced immune response and decreased tumorigenesis.

Other potentially immunosuppressive molecules

MUC1 glycoprotein is an epithelial associated mucin that is overexpressed, aberrantly glycosylated, shed from tumor cells during cancer transformation, and is implicated in the impairment of immune function in the vicinity of tumors. Depletion of soluble MUC1, by immunoprecipitation, from tumor supernatants neutralized the inhibitory effects on T cell proliferation [53]. Furthermore, human monocyte-derived DCs, when cultured in the presence of MUC1, displayed a modified phenotype with decreased expression of co-stimulatory molecules (CD86, CD40), antigen-presenting molecules (DR and CD1d), and differentiation markers (CD83) [54]. Several studies have shown MUC1 overexpression in PC and a correlation with advanced disease [55–57].

It is also suggested that PSA, found in high concentration in prostate tumor tissue, is immunosuppressive. One study demonstrated that PSA suppressed in vitro phytohemagglutinin- and alloantigen-stimulated lymphocyte proliferation in a dose-dependent manner [58]. Furthermore, the addition of active PSA to DC cultures significantly inhibited the generation and maturation of DC, as well as the ability of the DC to induce T cell proliferation [59].

Induction of T cell death

Fas ligand (FasL) is a type II transmembrane tumor necrosis factor family protein, known to trigger apoptosis in cells that bear the FasL receptor, Fas. The role of Fas in host-tumor interactions is complex, and Fas may have a beneficial role in the induction of tumor cell apoptosis, but may be a selective force influencing the escape of Fas-resistant aggressive tumor variants [60]. In PC patients soluble Fas levels are increased in serum [61], and LNCaP, DU145, and PC3 prostate carcinoma cells secrete soluble FasL into their local environment [62]. More recently, co-culture of T cells with exosomes derived from the LNCaP PC cell line dose-dependently inhibited T cell proliferation and induced apoptosis [63]. Addition of anti-FasL antibody blocked the T cell apoptosis induction by tumor exosomes. It is postulated that T cells themselves can commit suicide upon activation with antigen by secreting high amounts of FasL [64]. Indeed, TILs isolated from prostate tumors expressed higher amounts of FasL than their corresponding peripheral T cells [35]. However, the suggestion that immune cells can undergo apoptotic death at the tumor site through the Fas pathway has been received somewhat skeptically and awaits further studies to confirm this possibility [65].

T cell receptor dysfunction

T cells recognize antigen via the heterodimeric T cell receptor (TCR) molecule, which is noncovalently associated with the CD3 molecular complex. Reduced or aberrant expression of TCR-associated signal-transduction molecules is reported in many types of cancer and may contribute to tumor escape [10]. In PC, diminished expression of TCR-beta, CD3-epsilon, and CD3-zeta was observed on intratumoral T cells isolated from a TRAMP-C1P3 mouse model of PC [66]. Furthermore, expression of the zeta-chain was decreased in peripheral T cells of some PC patients, but normalized after treatment with a PSA vaccine [67, 68]. In addition to a functional TCR, a complex network of co-stimulatory and co-inhibitory signals regulate the T cell immune response [69]. Several studies in murine PC models have shown improved anti-tumor immune responses with the addition of co-stimulatory signals, such as B7.1 ligand, or with blockade of co-inhibitory signals, such as CTLA-4 [70–72]. Collectively, these data suggest that manipulation of T cell co-stimulatory and inhibitory signals may improve PC immunotherapy.

Tolerogenic dendritic cells

Dendritic cells (DCs) are phenotypically distinct and extremely potent antigen-presenting cells that are critical for initiation of specific immune responses [73]. DCs take up and process antigen as peptides within the major histocompatibility complex and present these to T cells. They also deliver additional co-stimulatory signals facilitating activation and expansion of antigen-specific T cells. Different types of DC exist which have different functional capacities with respect to the T cell responses that are induced, with tolerogenic immature and immunogenic mature differentiation stages [74]. In cancer, disabled DC differentiation, maturation, migration, and function have been proposed as one mechanism for tumor escape [75]. Indeed, in a murine model of PC, DCs isolated from TRAMP-C1P3 tumors were found to be phenotypically immature ($CD11c^{+}$, $B7.2^{+/-}$, $I\text{-}A^{-}$, $CD40^{-}$) [66]. Furthermore, in human prostate tumors DC were found to represent only a small subset of leukocytes present in both benign and malignant prostatic tissue. Statistically there were significantly less DC in PC compared with normal prostatic tissue, and these cells were minimally activated [76]. The presence of PC cells significantly inhibited the generation of DC, and these cells were weak stimulators of T cell proliferation, suggesting that DC generated in the PC microenvironment are functionally inhibited [77, 78]. Fortunately, many studies have demonstrated that good quality mature DCs can still be generated from the blood of PC patients, thus allowing their use as immunotherapeutic vaccines [79–81].

Regulatory T cells

Recently, it was suggested that the presence of regulatory $CD4^{+}CD25^{+}$ T cells may explain the poor clinical efficacy of immunotherapeutic protocols in human tumors [82]. It is now well established that immunoregulatory $CD4^{+}CD25^{+}$ T cells control key aspects of immunologic tolerance to self-antigens [83, 84]. These cells constitutively express CD25 (IL-2 receptor α-chain) on their surface and constitute 5–10% of $CD4^{+}$ T cells in humans and rodents. Although the precise mechanisms of suppression by Tregs remain to be determined, these cells inhibit immune-cell functions either directly through cell–cell contact or indirectly through the secretion of anti-inflammatory mediators, such as IL-10 and TGF-β.

Tien et al [85] demonstrated an increased frequency of tumor infiltrating $CD4^{+}CD25^{+}$ Tregs in the tumor mass of a 12T-7s murine spontaneous model of PC [85]. Furthermore, blockade of the $CD4^{+}CD25^{+}$ T cells using an anti-CD25 mAb reduced PC cell growth both in a prostate tumor transplant model (TRAMP-C2) and in the spontaneous prostate tumor model (12T-7s). Recently, we have found that $CD4^{+}CD25^{high}$ T cells are increased in the peripheral blood and tumor tissue of patients with early stage PC (unpublished data). Furthermore, in this study supernatants from primary prostate tumors, malignant ascites and PC cell lines, attracted $CD4^{+}CD25^{+}$ T cells in an in vitro migration assay and contained the chemokine CCL22, suggesting

one possible mechanism for the increased numbers of Tregs inside prostate tumors.

Concluding remarks

In general, cancer immunotherapy has not yet fulfilled its expectations and the knowledge of the immunobiology of PC is seriously lacking behind some other cancers, such as ovarian cancer and melanoma. Induction of tumor-specific T cells was demonstrated in some PC immunotherapy trials but it has been difficult to correlate this data with tumor regression. It is likely that a number of tumor escape pathways play a role in limiting the effectiveness of PC vaccines.

It is clear that vaccination alone will not be sufficient to create an effective immunotherapy for PC. An improved understanding of the mechanisms mediating tumor escape from immune recognition will lead to more effective vaccination protocols. Prospects for future investigation in PC could include combination of prostate-specific vaccines with low molecular weight inhibitors of immunosuppressive molecules or their receptors, improved immunological adjuvants, adoptive transfer of TCR transgenic T cells, or the removal of suppressive Tregs.

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