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Vaccines in urologic malignancies

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Abstract The prospect of activating the immune system to combat neoplastic disease has stimulated the interest of clinicians and scientists for over 100 years. Despite a few notable exceptions (especially with urologic malignancies), immunotherapy has not fully reached its considerable therapeutic potential for the treatment of cancer. Tumors undoubtedly express antigens that may act as targets for antitumor immunity, and advances in molecular biology and tumor immunology have recently revived the possibility of a cancer vaccine. This improved understanding has resulted in numerous successes with active immunotherapy in animal models and has facilitated the clinical testing of cancer vaccines. Ongoing advances in the identification of unique, tumor-specific antigens and their presentation to stimulate T cells will be necessary for the emergence of these novel vaccine therapies for cancer patients. Herein we review the current concepts of tumor immunology, including observations on cell types probably involved with the immune surveillance of tumors, the presentation and recognition of "foreign" antigens, and possible mechanisms of tumor escape from the immune response, all of which are critical to the understanding of new initiatives for cancer vaccine therapy. Finally, we review some of the successes and limitations of vaccine therapy for urologic malignancies.

Key words Immunotherapy · Genitourinary neoplasms · Tumor immunity

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Introduction

The ability to distinguish self from nonself is the hallmark of the immune system and facilitates our survival in an inordinately hostile environment. The human immune system not only allows the appropriate recognition and destruction of nonself but also the ability to recognize and tolerate self. Classically, vaccines have taken advantage of this phenomenon by stimulating a humoral response to specific bacterial or viral antigens to prevent disease caused by these infectious agents. The appeal of a vaccine for neoplastic cells is enormous and has been stimulated by immune surveillance theories whereby newly transformed cells are detected and eliminated by an intact immune response. Indeed, the study of tumor immunology is based on two simple propositions: (1) tumor cells express distinct antigens that are found in only negligible amounts in normal cells and (2) these antigens can be recognized by the immune system, leading to destruction of the "foreign" cell as though it were a transplant allograft or infectious agent.

Since the first description of the presence of tumor-associated antigens, numerous distinctive cell surface molecules have been identified that could be recognized as "foreign" by the host immune system. It therefore seems that for a tumor to establish itself and eventually to metastasize, it must first escape the surveillance of immune cells percolating throughout virtually all tissues of the body. Why surveillance of the immune system against tumors is not more effective is relatively poorly delineated, and investigation of these concepts has contributed immensely to our understanding of tumor development and may have important implications for cancer management. Several investigators interested in urologic malignancies including renal cell carcinoma and prostate cancer pioneered many of these early investigations. Subsequently, several tumor-associated antigens were identified for urologic cancers, although most of these targeted antigens are also found on normal cells (gp100, PSA, PSMA, etc.) or are develop-

mental antigens. Few truly foreign antigens have been identified.

Tumor immunology

The goal of tumor immunology is to understand the immune response to malignant cells and create novel therapeutic strategies. In humans, the concept of tumor surveillance by the immune system is somewhat vindicated by the increased occurrence of some rare tumors with chronic immunosuppression. Additionally, in rare cases of melanoma or renal cell carcinoma, spontaneous regressions prove the ability of the immune system to reduce tumor progression [61]. Furthermore, studies have demonstrated a reduced cell-mediated immunity in some cancer patients, and it has been observed that reduced NK cell cytotoxic activity may play an important role in prostate cancer development [49] and metastases [88]. Although this impaired cellular immunity can be demonstrated in cancer patients (especially those with advanced disease), it is likely that a generalized immunologic deficit is not causative but instead reflects a secondary phenomenon. In fact, the vast majority of cancers do not increase in immunodeficient hosts, suggesting that the normal immune response is unable to control most forms of cancer.

In the early twentieth century, Coley used heat-killed bacterial infections to initiate an antitumor response [32]. His observations led to the supposition that tumor escape mechanisms could be overridden to induce an antitumor response. However, clinically effective treatment failed to materialize until the advent of bacille Calmette-Guérin (BCG) for the treatment of superficial bladder cancer [50]. Rosenberg and associates revitalized interest in immunotherapy with their work on lymphokine-activated killer cells and tumor-infiltrating lymphocytes [3, 95]. Although therapeutic efficacy fell short of expectations, these studies demonstrate the ability of immune cells to eliminate tumors previously thought resistant to immune effector mechanisms. Gene therapy studies have also confirmed that most theoretically "nonimmunogenic" tumors are indeed immunogenic [5, 20, 99]. Nonetheless, numerous early studies demonstrated that specific immune cells recognize tumor-specific antigens, with resultant lytic activity of the tumor cells [80, 91]. In fact, a vast number of potential antigens result from any of several genetic alterations in cancer cells, including any amino acid sequence in any membrane-bound or intracellular protein.

Cell-mediated immunity

Since the discovery of tumor-specific transplantation antigens, cell-mediated immunity has been recognized as the predominant immune effector response in tumors, especially solid tumors. Cell populations involved in the immune response include lymphocytes, granulocytes, and specialized antigen-presenting cells (APC). The granulocytes include neutrophils which, along with macrophages and monocytes, are important for phagocytosis of antigens targeted by antibodies. The specialized APC include monocytes, macrophages, Langerhans' cells, Kupffer's cells, and dendritic cells. These cells express major histocompatibility complex (MHC) molecules classes I and II to display antigens appropriately to T lymphocytes (T cells). T cells, B lymphocytes (B cells), and natural killer (NK) cells make up the three major populations of lymphocytes and can be defined by the presence and type of transmembrane antigen receptors. The NK cells are large granular lymphocytes capable of killing certain cancer cells with no prior immunization and without restriction by MHC glycoproteins (which require T cells to recognize antigen in the context of self).

Although numerous cell types may be involved in antitumor immunity, including macrophages, antibody-dependent cell-mediated cytotoxicity (ADCC), and NK cells, evidence is emerging that T cells are most important in developing host antitumor activity. Mature T cells are comprised of two major populations defined by the cell surface expression of CD4 or CD8 molecules. The CD4+ cells recognize antigen when presented in association with MHC class II molecules, while CD8+ T cell (cytotoxic T cell or CTL) antigen recognition is restricted by MHC class I expression (Fig. 1).

T cells can also be classified by their functional role in modulating the immune response. Classically, CD4+ cells (helper T cells) have been thought to play an assisting role in stimulating other cells such as B cells, other T cells, and macrophages, whereas the CD8+ T cells are cytotoxic to cells displaying the antigen that they recognize. Furthermore, when naive T cells are activated, they produce interleukin-2 (IL-2) and then differentiate into two distinct subpopulations that can be categorized according to the different cytokines they produce [1].

The T helper subset, T_H1 cells, secrete predominantly IL-2, IFN-γ, and TNF-β and promote cell-mediated immune responses (i.e., delayed-type hypersensitivity, or DTH). During antigen presentation, APC produce IL-12, which drives T cells to differentiate toward a T_H1 response. IFN-γ secretion by T_H1 cells acts as an autocrine agonist and will inhibit differentiation of T_H2 cells, another T helper subset. T_H2 cells are the principle T helper cell for B cell function and propagation of the humoral allergic immune response. The cytokines they produce are predominantly IL-4, IL-5, IL-9, IL-10, and IL-13. IL-4 is known to enhance differentiation to a T_H2 response and at the same time antagonize the T_H1 response (Fig. 1).

The ability of the T cell to recognize antigen is a property of the T cell receptor (TCR) [11] and is specific for a certain peptide antigen/MHC combination. The TCR is a disulfide-linked heterodimer very similar in structure to the Fab fragment of an immunoglobulin molecule and is associated with signal-transducing

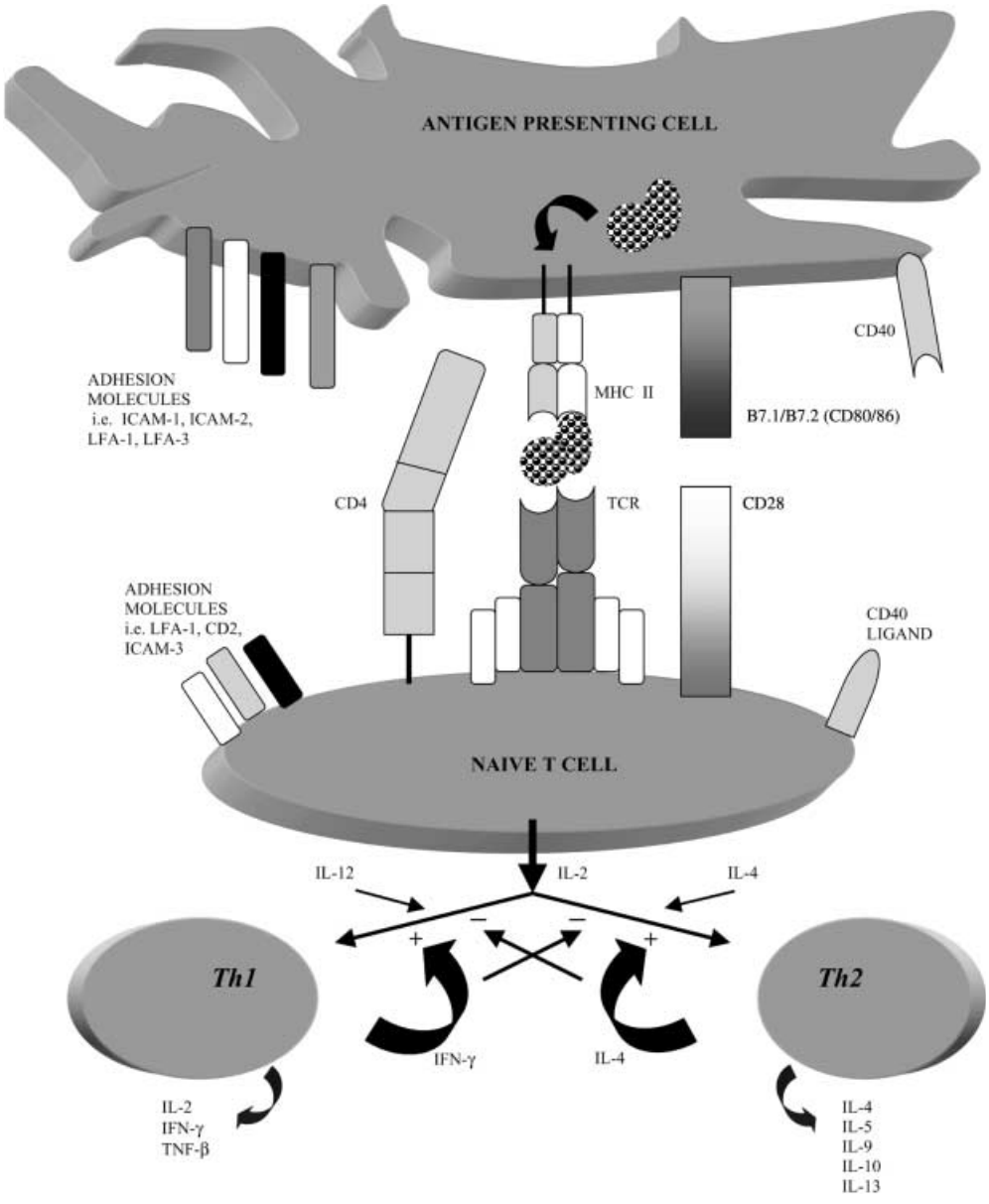

Fig. 1 Major histocompatability complex (MHC) class I molecules are found on virtually all nucleated cells and restrict antigen presentation to cytotoxic T cells. The MHC class II molecule is found only on the specialized antigen presenting cells such as macrophages, dendritic cells and B cells and restricts antigen presentation to helper T cells through the interaction with the CD4 molecule (as shown in the Fig. 1). Activation of T cells occurs after antigen presentation by the MHC molecules, and requires complex signaling by both the T cell receptor (TCR) and ligation of CD28 by the B7 molecules (CD80/CD86) on the APC. Another important costimulatory molecule is the CD40 molecule expressed on antigen presenting cells such as dendritic cells. Molecules necessary for cellular adhesion include LFA-1, CD2 and ICAM-3 on the T cell as well as ICAM-1, ICAM-2, LFA-1 and LFA-3 on the antigen presenting cells. Differentiation of T cells into T_H1 and T_H2 lymphocytes requires a complex interaction involving numerous immune cells and secreted cytokines, including IFN-γ secretion for T_H1 differentiation and IL-4 for T_H2 differentiation. The subsets are characterized by their secreted cytokine profile: IL-2, IFN-γ and TNF-β for T_H1 cells and IL-4, IL-5, IL-9, IL-10 and IL-13 for T_H2 cells. T_H1 subsets promote cell-mediated immune responses whereas T_H2 cells induce humoral-mediated allergic inflammation

molecules known as the CD3 complex [35]. The extracellular engagement of peptide antigen/MHC molecule to the TCR is insufficient to initiate the transduction of these intracellular signals. Important costimulatory molecules distinct from the TCR complex are also required for appropriate T cell activation. The most studied costimulatory signal is the ligation of a T cell surface molecule, CD28, to the CD80 (B7-1) or CD86 (B7-2) of the APC, as this is a requisite second signal for T cell activation [42]. Other cell surface molecules such as ICAM-3, LFA-1, and CD-2 are also important in T cell interactions with APC.

For T cells to recognize antigens and discriminate self from nonself, the antigenic peptides must be presented in association with major histocompatibility complex (MHC) molecules. The MHC is a genetic locus on chromosome 6 that encodes the cell surface structures, human leukocyte antigens (HLA) or H-2 in mouse.

There are two structural classes of HLA molecules, each of which restricts antigen recognition to different T cells. The three-dimensional structure of the class I and II molecules exposes a groove involving 8 to 25 amino acids that can be recognized by the TCR of the T cells. With the aid of the MHC molecules, T cells can recognize foreign antigens in the context of self, as the TCR recognizes a composite of antigens associated with the correct MHC molecule.

Class I HLA molecules are found on virtually all nucleated cells and restrict CTL. They are composed of a 44-kd α chain encoded within the MHC. This glycoprotein is associated with β2-microglobulin encoded by a non-HLA gene found on chromosome 15 [12]. HLA-A, -B, and -C are the three major class I genes and numerous alleles are defined for each of them [100]. Class II molecules are also heterodimeric structures, although they are found only on specialized antigen-presenting cells such as macrophages, dendritic cells, and B cells and restrict antigen recognition to T helper cells. There are also only three major categories of class II molecules, HLA-DR, -DQ, and -DP, and each molecule is made up of α and β chains encoded within the MHC [14].

Tumor escape mechanisms

Why the immune surveillance is not more effective in controlling tumor formation/progression is poorly understood. One possible mechanism of tumor escape is the selection of tumor cell clones expressing fewer immunodominant antigens by the pressure of normal host immune surveillance. Several studies have documented the outgrowth of tumor cell lines with few tumor-specific antigens, and this loss of antigen expression could be due to antibody-induced internalization or antigenic variation. Fortunately, even immunoselected tumor cell variants have been shown to express a number of unique antigens that could serve as targets for immunotherapy protocols [23].

Even if stable molecules are present, the tumor-bearing host may become tolerant to these antigens. This tolerance may be due to improper antigen presentation or neonatal exposure to the antigen, as in the case of carcinoembryonic antigen (CEA). However, studies have demonstrated that immunization with some of these antigens can overcome tolerance and subsequently may be used for immunotherapy regimens [60].

Downregulation of the MHC molecules may also lead to tumor escape, as the lack of MHC input would result in ineffective presentation of antigen to immune cells in the context of self and may lead to suppression of the appropriate immune response. Downregulation of MHC class I molecules often increases tumorigenesis in animal models. Wallich showed that transfecting the MHC class I genes can inhibit the metastatic ability of tumor cells [105]. Bander et al. has confirmed lost or diminished class I expression in a number of cell lines as well as in frozen tissue specimens of prostate cancer [7]. Levin demonstrated that less differentiated prostate tumors expressed significantly fewer MHC class I molecules and, when both class I expression and degree of differentiation were considered, those with higher expression had better survival [55]. The authors suggest that HLA class I status may be an important prognostic factor as well as an important target for future immunotherapy strategies.

As discussed earlier, effective CTL activation is dependent on stimulatory signals from the T_H1 subpopulation of helper T cells. As most solid tumors do not express class II MHC molecules necessary for antigen presentation to the helper T cells, tumor development may take advantage of the lack of activating signals. Recent studies have revealed that in a number of advanced malignancies, the zeta (ζ) chain of the T cell receptor in tumor-infiltrating lymphocytes shows decreased expression [25] and that this loss is associated with poorer prognosis [111]. Healy similarly demonstrated impaired expression and function of ζ chains in the peripheral blood lymphocytes in patients with advanced prostate cancer [36]. A number of molecules have been shown to be critical for effective activation of T cells, and the lack of these costimulatory signals may lead to tolerance of T lymphocyte responses. The best characterized of these costimulatory molecules is the B7-CD28 receptor ligand pair [54], which has been shown in animal studies to augment the antitumor immune response when amplified. Other molecules necessary for the binding of lymphocytes to antigen-presenting cells include LFA-1, CD2, and ICAM-3 on T cells as well as ICAM-1, ICAM-2, LFA-1, and LFA-3 on the antigen-presenting cells.

The resistance of certain populations of neoplastic cells to cytotoxic cell killing mechanisms may be another mechanism of tumor escape. The inability of tumor cells to undergo Fas-mediated death may contribute to evasion of immune surveillance. Lehmann et al. recently described a novel method of tumor escape to NK-mediated killing. They describe the impaired binding of perforin on the cell surface of tumor cells, with subsequent resistance of granule-mediated cell death from the cytotoxic effector cells [53].

Investigators have demonstrated that tumor cells can further affect their environment to potentially decrease the effectiveness of any host antitumor immune response. Examples include the release of free antigens that can interfere with the effective response of NK cells and helper T cells. Also, tumor cell production of cytokines such as IL-10 and IL-18 has been shown to limit the effectiveness of immune surveillance. Transforming growth factor-β (TGF-β) has been demonstrated to inhibit IL-2-dependent immune responses of macrophages and T cells [26]. Another mechanism of tumor escape may involve Fas/Fas ligand interaction. Several human tumors have been found to express Fas ligand, which can induce Fas receptor-mediated apoptosis of activated immune cells [10]. Kim and associates found high

expression of Fas ligand and a relatively low expression of Fas in renal cell carcinoma, which may be involved in the evasion of immune effector cells, although no correlation could be found with tumor stage or grade [47].

Finally, it is possible that host cells can be recruited to the tumor site for active downregulation of antitumor T cell immune responses [74]. There is some experimental evidence for the existence of T cell subsets that may play an antigen-specific suppressor role in tumor immunology. These T cells have been shown to be CD4+ and may have T_H2 characteristics, although their role in human cancers has been difficult to determine.

Tumor immunotherapy

Numerous therapeutic strategies utilizing the immune system to eradicate neoplastic disease have been developed (Table 1). Early attempts focused on nonspecific strategies and the best example of cancer immunotherapy is still the in situ use of bacille Calmette-Guérin (BCG) for carcinoma of the bladder. Although the exact antitumor mechanism has still not been completely delineated, it undoubtedly relies on a functioning immune system [75] and, in particular, requires the presence of both CD4+ and CD8+ T cell subsets [76].

Adoptive immunotherapy is a passive, nonspecific technique involving the transfer of immunologically activated lymphoid cells. Clinical experience has revealed that the activation of human lymphoid cells, i.e., IL-2-activated LAK cells (TIL), is feasible and that systemic administration is safe; however, clinical success in protocols for renal cell carcinoma and melanoma has been limited [3, 95]. Lubaroff et al. [56] have shown that a severe combined immunodeficiency (SCID) mouse model is a viable system for studying adoptive therapies for human prostate cancer and, furthermore, demonstrated antitumor activity utilizing autologous IL-2-activated tumor-infiltrating lymphocytes (personal communication). A novel approach to adoptive therapy was reported by Cesano et al. whereby the MHC nonrestricted human T cell line TALL-104 demonstrated a significant antitumor effect against DU-145 tumors in SCID mice [16].

The systemic administration of cytokines such as IL-2, IL-4, and recently IL-12 is an example of active nonspecific immunotherapy. The first extensive studies of cytokine immunotherapy were performed with IL-2, and remissions reported in patients with renal cell carcinoma and melanoma give a "proof of principle" of tumor immunotherapy, even for metastatic disease. IL-12, a heterodimeric cytokine with immunoregulatory activity, has shown potent antitumor activity in a number of different murine tumor models. However, enthusiasm for these systemic cytokine therapies needs to be tempered by the frequent reports of severe toxic effects.

Cancer vaccines

An alternative immunotherapeutic strategy to simple systemic administration of cytokines or activated immune cell populations is generation of a vaccine that could elicit a specific antitumor response in vivo. In this regard, there are a number of significant differences in the development of vaccines against infectious agents as opposed to neoplastic cells. Viral genes, for example, are relatively simple, dictating a limited number of defined antigens to be manipulated for immunization. Tumor cells, on the other hand, possess a virtually unlimited number of antigens, the majority of which have not been identified. Vaccination against infectious agents occurs prior to exposure in order to prevent disease, whereas in the case of a cancer vaccine, the immune system has

Table 1 Immunotherapy strategies

Type	Description	Example
Passive immunotherapy		
Anti-tumor antibodies	Administration of tumor-specific antibodies	Radioisotope-labeled anti-PSMA
	Complexed with toxic materials, drugs, radioisotopes	Anti-PSA carrying chemotherapeutics
Adoptive immunotherapy	Transfer of activated lymphoid cells	IL-2 activated LAK cells
		Human T cell line TALL-104
Active immunotherapy		
Cytokines	Systemic administration of cytokines to stimulate an antitumor response	IL-2 for renal cell carcinoma
		IL-4, IL-12
Tumor vaccines		
Antigen-specific	Recombinant peptide stimulating an antitumor response delivered as	PSA/PSA segment vaccine
	– DNA vaccine	Vaccinia virus/PSA vaccine
	– Peptide/protein vaccine	Dendritic cell/PSMA vaccine
	– Viral or bacterial vaccine	
	– Peptide-pulsed dendritic cell vaccine	
Tumor cell vaccine	Ex vivo administration of modified autologous or allogeneic tumor cells	Autologous prostate tumor cell vaccine
	In situ modification of tumors to stimulate antitumor response	In situ transfer of cytokines to tumors, i.e., IL-2 and IL-12

theoretically already been exposed to the tumor antigens and therefore may already be "tolerant" to them. Finally, the majority of cancer vaccines focus on stimulating a different arm of the immune response, cell-mediated immunity, not the usually desired humoral response to infectious agents.

There are generally two ways of categorizing cancer vaccines, depending on the source of the immunizing antigens: antigen-specific or cell-based cancer vaccines [34].

Antigen-specific vaccines

Antigen-specific vaccines involve the delivery of recombinant peptides or proteins to a host in order to elicit an antitumor immune response. Much interest has been stimulated in this possibility since the first convincing demonstration of the existence of specific tumor-associated antigens [72], and the development of several novel techniques to define MHC class I-restricted antigens molecularly has facilitated an explosion of research in this area [13, 15, 37, 40, 46, 66, 70, 79]. Any immunotherapy protocol has several different means of delivering these antigens to the host:

1. DNA-based vaccines
2. Peptide- or protein-based vaccines
3. Recombinant viral or bacterial vaccines
4. Antigen-pulsed dendritic cell vaccines

DNA vaccines encoding tumor antigens can be delivered encapsulated by liposomes or as naked DNA [66]. Despite the possibility of rapid degradation of DNA when given systemically, there has been renewed interest in DNA-based vaccines for both infectious agents and neoplastic diseases [96, 108]. Xiang et al. recently described an autologous oral DNA vaccine delivered with an attenuated strain of *Salmonella typhimurium* to immunize mice successfully in a self-antigen model of melanoma [108].

The advantages of administering a cancer vaccine as a peptide or protein would include greater safety than with other vector-based vaccines. Tumor antigens could be delivered simply as defined peptides or as proteins specifically designed to access the class I pathway of APC in vivo. Several phase I clinical trials have been completed utilizing peptide-based vaccines for malignant melanoma, demonstrating minimal toxicity and some clinical and in vitro evidence of response [58, 71, 83].

Advances in recombinant technology have stimulated interest in the incorporation of genes encoding relevant antigens into vectors such as bacteria or viruses to augment their immune response. Vectors such as *Listeria* monocytogenes, adenovirus, and the pox viruses (including vaccinia) impart a number advantages including high-efficiency gene transfer and targeting of the MHC class I and II antigen-processing pathways [39, 43, 69, 78, 112].

While viruses are effective in generating CTL and ultimately antitumor activity in naive mice, the use of viruses in settings where the host has been previously exposed to the virus significantly reduces transgene expression [48, 109]. Studies by Yang and associates formally demonstrated that T cell responses, specifically of CTL, to viral proteins were responsible for destruction of cells expressing the transgene [109]. Furthermore, antibodies in the serum have been shown to reduce the ability of viruses to deliver the transgene [48], and the resulting decrease in gene expression reduces subsequent CTL activation [41, 63]. The diminished ability to generate CTL when antibodies to the viral vector are present has been suggested as an important reason for the lack of CTL after adenovirus delivery of melanoma antigens in clinical trials [84].

To facilitate the presentation of antigenic peptides as a cancer vaccine, much interest has focused on the use of dendritic cells. These bone marrow-derived cells have the ability to process antigens and present them in the context of MHC molecules and other costimulatory molecules such as B7. They are the most potent APC identified and are capable of activating naive T cells. A potentially powerful strategy in cancer gene therapy involves the genetic engineering of dendritic cells with defined tumor antigens and their use as vaccines [97]. Dendritic cell vaccines can be produced by loading these cells in vitro with peptides, proteins, and whole tumor cells or by infecting them ex vivo by viral vectors [94]. The first few clinical trials utilizing a dendritic cell-based vaccine for metastatic melanoma and follicular B-cell lymphoma have demonstrated immunologic and clinical response [38, 65].

Tumor cell vaccines

Active immunization can also be achieved by the administration of autologous or allogenic tumor cells modified to secrete cytokines or other immunostimulatory molecules capable of recruiting antitumor effector cells. Examples include in-vitro tumor cell transfection by MHC class II genes [68], transfection of lost MHC class I alleles [105], and transfection of the costimulatory molecule B7 to stimulate T cells [8]. The strategy of using tumor cell vaccines as a source of tumor antigens eliminates the need to identify and develop immunodominant antigens. Engineered tumor cells expressing cytokines may function as well by providing necessary growth factors to activated CTL in the absence of helper T cells. Numerous different cytokines have been introduced into tumor cells in vitro, including IL-2, IL-4, IL-6, IL-12, TNF-α, and GM-CSF. Many of these preclinical models have shown great success in controlling local tumor outgrowth as well as some measure of systemic protection. Numerous phase I/II clinical trials utilizing ex vivo transduced whole-tumor cell vaccines (most often involving malignant melanoma) have been published [22, 29, 90].

Cancer gene therapy strategies involving the transfer of genetic material in situ by viral and nonviral vectors

have also been successful in stimulating active antitumor immunization. These in vivo schemes offer a number of practical advantages over their ex vivo counterparts, including the cost reduction of bypassing the isolation of target cells from the patient. Putzer demonstrated impressive regression of preestablished tumors through the intratumoral injection of adenovirus recombinant for IL-12 and the B7-1 molecule [73]. We have also observed significant antitumor activity of murine prostate cancer nodules after intratumoral injection of the canarypox virus, ALVAC, recombinant for murine IL-2, IL-12, and TNF-α.

Vaccines in urologic malignancies

Immunotherapy for urologic malignancies has a rich history, given its arguably gold-standard applications in bladder and renal cancer. Advances in molecular techniques and knowledge of the immune response to tumor cells have generated great enthusiasm in the urologic community to the possibility of novel alternative or adjuvant approaches to the neoplastic diseases that we treat.

Prostate cancer

Despite the enormous prevalence of prostate cancer, its optimal management remains at best controversial, with 5-year biochemical failure rates for radical prostatectomy ranging from 27% to 57%. It is imperative to develop alternative or adjuvant treatment strategies, and immunotherapy presents a unique opportunity for better management of clinically localized and metastatic disease.

Prostate cancer is truly an exciting model for immunotherapy trials. Not only do numerous animal models exist, but human prostate cancer has also shown a variety of tumor-associated antigens. A few examples of these are PSA, hK2, PSCA, PSMA [40], MUC-1 [15], MUC-2 [28], GLOBO-H [46], GM-2 [45], and Lewis Y [37], some of which may well serve as targets for antigen-specific immune recognition [93]. Segments of the PSA protein have been shown to be immunogenic and stimulate a specific CTL reaction when used as a vaccine. Several peptide antigens, two 10-mer peptides from PSA (PSA-1 and PSA-3), and a PSA oligoepitope peptide (PSA-OP), have also been shown to elicit CTL responses in vitro [21]. From this initial work, several phase I/II clinical studies are underway in which patients are vaccinated against the PSA protein with the hope that systemic immunity against prostate cancer will occur. Phase I clinical trials have also been performed using autologous dendritic cells associated with the HLA-2.1-specific prostate-specific membrane antigen (PSMA). Little adverse effect was observed and several hormone-refractory patients showed significant responses to the vaccine [86]. The phase II trial using this dendritic cell vaccine demonstrated an overall response rate of 30% (19/62), with 11 durable responses [98].

Several investigators have developed novel tumor cell vaccine strategies to treat prostate cancer. Early studies by Sanda and associates showed that GM-CSF-transfected rat prostatic adenocarcinomas grew more slowly than parental tumors [87]. Subsequently, Vieweg showed that IL-2 transfected rat R3327-MatLyLu also induced antitumor activity [102]. Existing tumors had reduced rates of tumor outgrowth, and protection was gained against subsequent tumor challenge. Using the parental R3327G tumor that exhibits hormone responsiveness, Yoshimura also observed antitumor activity; however, neither CTL activity nor protection against subsequent tumor challenge was observed [110]. We reported one approach to cancer immunotherapy involving the transfer of genes encoding the cytokines IL-2 and TNF-α using the canarypox viral vector, ALVAC [44]. The ALVAC virus was shown to infect efficiently murine prostate cancer cells, RM-1, and produce high levels of extrinsic gene product. Antitumor immunity was also induced when tumor cells were infected by ALVAC cytokine recombinants and injected subcutaneously in the flanks of male C57BL/6 mice. Based on the optimistic results of such preclinical studies, an autologous vaccine approach is being investigated in a phase I/II study at Johns Hopkins Clinic in patients with extracapsular disease following radical prostatectomy. An allogeneic vaccine using MHC class I-matched allogeneic cells transduced to secrete IL-2 and IFN-γ is being employed in a NCI phase I/II study at the Memorial Sloan Kettering Institute. Likewise, a phase I clinical protocol for the intratumoral (in situ) injection of IL-2 and GMCSF genes is underway for prostate cancer.

Kidney cancer

Management of metastatic renal cell carcinoma (RCC) has provided a "proof of principle" for immunotherapeutic approaches to the treatment of cancer. Metastatic RCC is a refractory disease that typically responds poorly to other interventions including radiotherapy and chemotherapy. However, systemic administration of cytokines such as IL-2 and interferon-α resulted in response rates from 15% to 25% [82, 89, 104, 107]. Further evidence for the ability of RCC to act as targets for immunomodulatory techniques include the clinical response rates to adoptive immunotherapy utilizing lymphokine-activated killer cells and tumor-infiltrating lymphocytes [9, 24, 81]. Similarly, Childs et al. recently described graft-vs-tumor responses after nonmyeloablative allogenic stem cell transplantation [19].

A recently described tumor marker for renal cell carcinoma, G250 antigen, has potential as a target for an antigen-specific vaccine-based therapy. It is a transmembrane protein identical to the tumor-associated antigen MN/CAIX [33] and highly expressed on kidney cancer cells, with little expression in normal cells [6]. This antigen has been a target for immunohistochemical studies [4] as well as a radioimmunotherapeutic clinical

trial [106]. The use of G250 as a target for vaccine therapies has been investigated in several preclinical reports. Van Dijk et al. reported on the enhancement of an anti-tumor immune response in a xenogenic model of renal cell carcinoma using the administration of antibodies to G250 [101]. Subsequently, Vissers et al. have analyzed the immunogenicity of G250 epitopes and concluded that this antigen may well serve as an important target for anti-RCC immunotherapy [103].

Despite the identification of G250 as an organ-specific antigen, relatively few tumor-associated antigens have been described for RCC. Instead, numerous reports have looked at the potential of using whole tumor cells as a source of potential antigens [2, 27, 59, 77, 85]. These clinical studies employed autologous allogenic tumor cells injected with immune adjuvants such as BCG, *C. parvum*, and *Candida* antigen. Although specific conclusions are difficult to ascertain based on the marked differences in these trials, it is encouraging that evidence of some clinical tumor regression or immune activation was consistently observed. Galligioni et al. [27] initiated a clinical trial utilizing an autologous tumor cell vaccine in patients after radical nephrectomy. The tumor cells were injected intradermally with BCG as an adjuvant. Although a survival benefit was not observed, significant differences in the DTH response to the tumor cells compared to normal renal cells did suggest the presence of a tumor-specific cell-mediated immune response.

Numerous technical advances have facilitated our ability to improve the immunogenicity of tumor cells with the transfer of genes encoding various cytokines such as TNF, IL-2, IL-4, and GM-CSF as well as costimulatory molecules such as B7 [30, 31, 92]. In this regard, Gansbacher et al. [30] reported on a pilot study of an allogeneic vaccine secreting IL-2, and Simons et al. [92] demonstrated provocative results with an autologous GM-CSF-secreting vaccine for patients with metastatic renal cell carcinoma.

Bladder cancer

Since the first instillation of BCG into the bladders of patients with superficial TCC [62], it has become increasingly clear that bladder cancer is the most immunosensitive tumor and an ideal candidate for future immunotherapy research. The observations confirming the role of an intact immune response in the mechanism of antitumor activity for BCG have stimulated several trials utilizing immunomodulating techniques. Some of these include the intravesical instillation of cytokines such as IL-2 and the interferons. O'Donnell et al. reported on the safety and efficacy of a modified BCG that expresses the gene for IL-2 [18, 67].

Although several tumor-associated proteins have been identified for bladder cancer, most were investigated as biologic markers for the detection of disease and not as possible targets for a vaccine strategy. However, Luo et al. identified a mutated form of ras p21 protein in the MB49 murine cell line, and immunization resulted in mutation-specific killing of tumor cells [57]. Nair et al. employed an antigen-specific strategy by vaccinating mice with dendritic cells pulsed with MBT-2 tumor extracts [64]. They were able to demonstrate cytolytic activity in vitro and resistance to subsequent tumor rechallenge.

Several investigators are studying the transfer of genes to bladder cancer cells in order to establish a whole-tumor cell vaccine for treating localized and systemic disease. Larchian et al. described a tumor cell vaccine in an orthotopic murine model in which cells transfected with IL-2 or B7 genes were administered systemically [51]. This approach resulted in significant tumor regression and survival benefit, especially in mice treated with the combined vaccine (IL-2 and B7). Connor et al. has also successfully used IL-2 gene transfection as a cancer vaccine strategy for therapy in a murine model of bladder cell carcinoma [20]. Chen et al. used a recombinant adenoviral vector to transfer the IL-12 gene to the MB49 cell line and demonstrated significant tumor inhibition as well as subsequent specific protective immunity [17].

Several studies have also looked at the in vivo transduction of tumor cells to induce an immune response as an in situ tumor cell vaccine. Based on the easy access to the relatively nonabsorptive genitourinary epithelium as well as the vast experience with intravesical treatments for several bladder diseases, many consider bladder cancer to be an attractive candidate for such strategies. In this regard, Lee et al. [52] have demonstrated infectivity of murine and human transitional cell lines by a recombinant vaccinia vector that may be optimally suited for an immunotherapy protocol.

Conclusions

Dramatic advances in available molecular techniques and growing understanding of antigen presentation and recognition have led to much renewed interest in immunotherapy as a possible adjuvant or alternative therapy for cancer. Those investigating such possibilities for urologic malignancies have been leaders in the field. Novel preclinical studies and early clinical trials have resulted in numerous successes; however, we should take care to interpret this data cautiously as tumor immunotherapy emerges from its infancy.

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