

Review Article

Immunologic Monitoring of Cellular Responses by Dendritic/Tumor Cell Fusion Vaccines

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Although dendritic cell (DC)-based cancer vaccines induce effective antitumor activities in murine models, only limited therapeutic results have been obtained in clinical trials. As cancer vaccines induce antitumor activities by eliciting or modifying immune responses in patients with cancer, the Response Evaluation Criteria in Solid Tumors (RECIST) and WHO criteria, designed to detect early effects of cytotoxic chemotherapy in solid tumors, may not provide a complete assessment of cancer vaccines. The problem may, in part, be resolved by carrying out immunologic cellular monitoring, which is one prerequisite for rational development of cancer vaccines. In this review, we will discuss immunologic monitoring of cellular responses for the evaluation of cancer vaccines including fusions of DC and whole tumor cell.

1. Introduction

The mechanism of action for most cancer vaccines is mainly mediated through cytotoxic T lymphocytes (CTLs). We are now gaining a clear understanding of the cellular events leading to an effective CTL-mediated antitumor immunity. The antigen-presenting cells (APCs) most suitable for cancer vaccines are dendritic cells (DCs), which can be distinguished from B cells and macrophages by their abundant expression of costimulatory molecules and abilities to initiate a strong primary immune response [1, 2]. DCs are specialized to capture and process tumor-associated antigens (TAAs), converting the proteins to peptides that are presented on major histocompatibility complex (MHC) class I and class II molecules [3]. After TAAs uptake and inflammatory stimulation, immature DCs in peripheral tissues undergo a maturation process characterized by the upregulation of costimulatory molecules [2, 3]. During this process, mature DCs migrate to T-cell areas of secondary lymphoid organs, where

they present antigenic peptides to CD8+ and CD4+ T cells through MHC class I and class II pathways, respectively, and become competent to present antigens to T cells, thus initiating antigen-specific CTL responses [4]. Antigen-specific CTLs in turn can attack tumor cells that express cognate antigenic determinants or can provide help for B-cell responses that produce antibodies, which can also lead to tumor cell death in some cases [5]. Thus, the mechanism of action for cancer vaccines, based on harnessing host immune cells to infiltrate tumors and to exert CTL responses, is quite different from that of a traditional cytotoxic chemotherapy [6].

2. DC-Based Cancer Vaccines

A major area of investigation in induction of antitumor immunity involves the design of DC-based cancer vaccines [7]. DCs derive their potency from constitutive and inducible expression of essential costimulatory molecules including

B7, ICAM-1, LFA-1, LFA-3, and CD40 on the cell surface [1, 8, 9]. These proteins function in concert to generate a network of secondary signals essential for reinforcing the primary antigen-specific signals in T-cell activation. Therefore, many strategies have been developed to load TAAs onto DCs and used as cancer vaccines. For example, DCs are pulsed with synthetic peptides derived from the known antigens [10], tumor lysates [11], tumor RNA [12, 13], and dying tumor cells [14] to induce antigen-specific antitumor immunity. Although the production of DC-based cancer vaccines for individual patients with cancer has currently been addressed in clinical trials, a major drawback of these strategies comes from the limited number of known antigenic peptides available in many HLA contexts. Moreover, the results of clinical trials using DCs pulsed with antigen-specific peptides show that clinical responses have been found in a small number of patients [15, 16]. To overcome this limitation, we have proposed the fusions of DCs and whole tumor cell (DC/tumor) to generate cell hybrids with the characteristics of APCs able to process endogenously provided whole TAAs [17]. The whole tumor cells may be postulated to serve as the best source of antigens [17–21].

3. DC/Tumor Fusion Vaccines

The fusion of DC and tumor cell through chemical [17], physical [22], or biological means [23] creates a heterokaryon which combines DC-derived costimulatory molecules, efficient antigen-processing and -presentation machinery, and an abundance of tumor-derived antigens including those yet to be unidentified (Figure 1). Thus, the DC/tumor fusion cells combine the essential elements for presenting tumor antigens to host immune cells and for inducing effective antitumor responses. Now, it is becoming clear that the tumor antigens are processed along the endogenous pathway, through the antigen processing machinery of human DC. Thus, it is conceivable that tumor antigens synthesized *de novo* in the heterokaryon are processed and presented through the endogenous pathway. The advantage of DC/tumor fusion vaccines over pulsing DC with whole tumor lysates is that endogenously synthesized antigens have better access to MHC class I pathway [18–21]. Indeed, it has been demonstrated that DC/tumor fusion vaccines are superior to those involving other methods of DC loaded with antigenic proteins, peptides, tumor cell lysates, or irradiated tumor cells in murine models [18–21]. The efficacy of antitumor immunity induced by DC/tumor fusion vaccines has been demonstrated in murine models using melanoma [24–32], colorectal [17, 30, 31, 33–41], breast [42–47], esophageal [48], pancreatic [49, 50], hepatocellular [51–55], lung [22, 41, 56–59], renal cell [60] carcinoma, sarcoma [61–66], myeloma [67–74], mastocytoma [75], lymphoma [76], and neuroblastoma [77]. The fusion cells generated with human DC and tumor cell also have the ability to present multiple tumor antigens, thus increasing the frequency of responding T cells and maximizing antitumor immunity capable of killing tumor targets such as colon [78–84], gastric [85, 86], pancreatic [87], breast [47, 88–93], laryngeal [94], ovarian [95–97], lung [85, 98], prostate [99, 100], renal cell

[101, 102], hepatocellular [103–105] carcinoma, leukemia [106–111], myeloma [112, 113], sarcoma [114, 115], melanoma [116–119], glioma [120], and plasmacytoma [121].

4. Monitoring of DC/Tumor Fusion Cell Preparations

Despite the strong preclinical evidences supporting the use of DC/tumor fusions for cancer vaccination, the results of clinical trials so far reported are conflicting [18–21]. One of the reasons is the evidence for fusion cell formation used as clinical trials is not definitive [23]. The levels of fusion efficiency, which can be quantified by determining the percentage of cells that coexpress tumor and DC antigens, are closely correlated with CTL induction *in vitro* [82, 83]. Another reason is immunosuppressive substances such as TGF- β derived from tumor cells used for fusion cell preparations [35, 47]. Although tumor-derived TGF- β reduces the efficacy of DC/tumor fusion vaccines via an *in vivo* mechanism [35], the reduction of TGF- β derived from the fusions inhibits the generation of Tregs and enhances antitumor immunity [47]. Moreover, the therapeutic effects in patients vaccinated by DC/tumor fusions are correlated with the characteristics of the DCs used as the fusion vaccines [82, 83]. Indeed, patient-derived fusions show inferior levels of MHC class II and costimulatory molecules and produce decreased levels of IL-12 and increased levels of IL-10, as compared with those obtained from fusions of tumor cell and DC from healthy donors [87, 103]. However, the fusion vaccines induce recovery of DC function in metastatic cancer patients [103]. Therefore, it is important to assess the phenotype and function of DC/tumor fusion cell preparations used in each vaccination.

5. In Vivo Monitoring

The delayed-type hypersensitivity (DTH) is an inflammatory reaction mainly mediated by CD4+ effector memory T cells that infiltrate the site of injection of an antigen against which the immune system has been primed by cancer vaccines [122]. Actually, soluble proteins, peptides, or antigens pulsed DCs have been injected intradermally, and the diameter of erythema or induration after 48–72 h is measured. CD4+ effector memory T cells that recognize the antigens presented on local APCs mediate the immune responses by releasing cytokines, resulting in an increased vascular permeability and the recruitment of monocytes and other inflammatory cells in the site. CD8+ T cells less frequently also mediate similar responses [123]. It has been reported that antigen-specific T cells can be readily detected in skin biopsies from DTH sites, much less in abdominal lymph nodes, and not in peripheral blood and tumor site [124]. Moreover, there is a significant correlation between favorable clinical outcome and the presence of vaccine-related antigen-specific T cells in biopsies from DTH sites [122]. Indeed, the increased DTH reactivity against tumor antigens has been observed in clinical responders by DC/tumor fusion vaccines [125]. In almost patients with cancer, T cells from lymph nodes and the tumor site itself are not readily available for

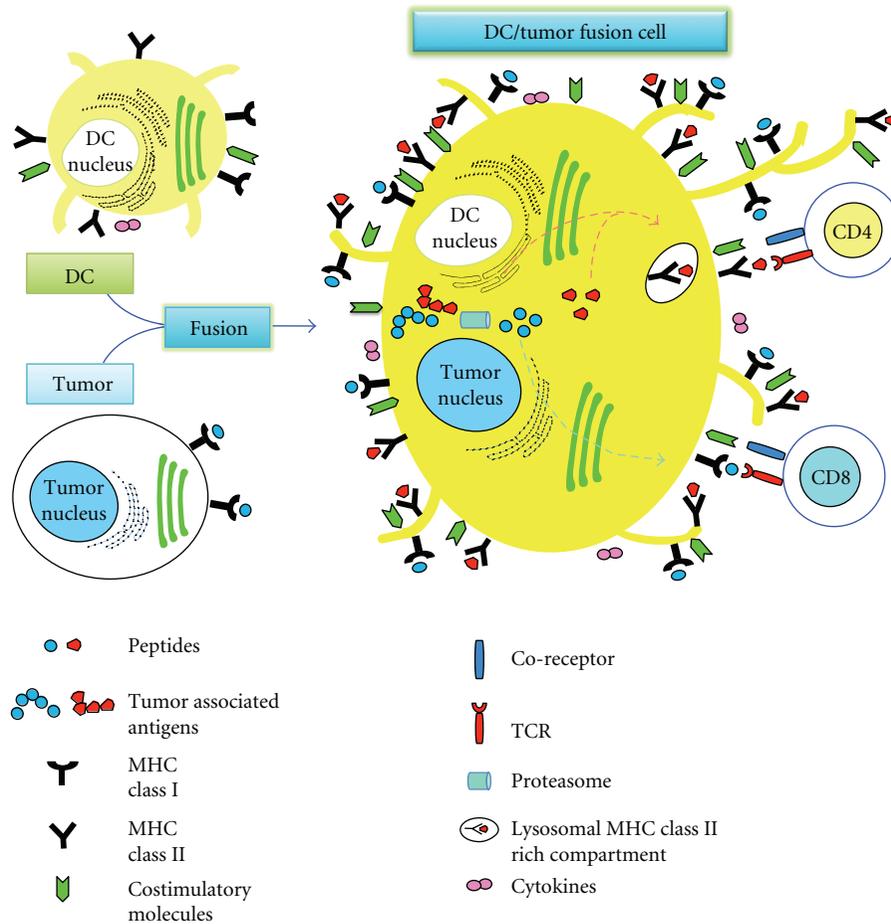


FIGURE 1: A model of antigen processing and presentation by DC/tumor fusion cell. DC/tumor fusion cell expresses MHC class I, class II, costimulatory molecules, and tumor-associated antigens. Tumor-associated antigens can be processed and presented through the antigen processing and presentation pathway of DC.

monitoring purposes. Therefore, functional assessment of antigen-specific T cells from such DTH sites may serve as an additional strategy to evaluate antigen-specific antitumor immune responses [122, 126, 127].

6. T-Cell Monitoring In Vitro

The mechanism of cancer vaccines, based on inducing CTLs, infiltrating tumors, and exerting T-cell-mediated cytotoxic effects, is quite different from that of cytotoxic chemotherapy. As cancer vaccines do not work as quickly as chemotherapy which has a direct cytotoxic effect, the Response Evaluation Criteria in Solid Tumors (RECIST) and WHO criteria [128, 129], designed to detect early effects of cytotoxic chemotherapy, cannot appropriately evaluate the response patterns observed with cancer vaccines. The RECIST criteria are highly dependent upon measurement of tumor size. They presume that linear measures are an adequate substitute for 2-dimensional methods and register four response categories: CR (complete response), PR (partial response), SD (stable disease), and PD (progressive disease). However, in the solid tumors, there exist not

only antigen-specific CTLs but also immune suppressive cells such as myeloid-derived suppressor cells (MDSCs) [130], immunosuppressive tumor-associated macrophages (TAMs) [131], and cancer associated fibroblasts (CAFs) [132] (Figure 2). After vaccination, the solid tumors may become heavily infiltrated by immune-related cells resulting in an apparent increase in size of lesions, which is, at least in part, due to the infiltration of CTLs induced by cancer vaccines. Therefore, the development of new response criteria, including immunologic cellular monitoring, is of great importance in the development of cancer vaccines.

In clinical trials, the peripheral blood T-cell responses are generally accessible for serial analyses. The currently used methods of assessing T-cells from patients treated with cancer vaccines are T-cell proliferation, cytokine profile, cytotoxic T lymphocyte assays (CTL assays), CTL-associated molecules (CD107, perforin, granzyme B, and CD154), multimer analysis, T-cell receptor (TCR) gene usage, and immune suppression assays (Table 1). While these assays can be also used for monitoring cellular immune responses induced by DC/tumor fusion vaccines, none has been standardized. As DC/tumor fusion vaccines can induce defined

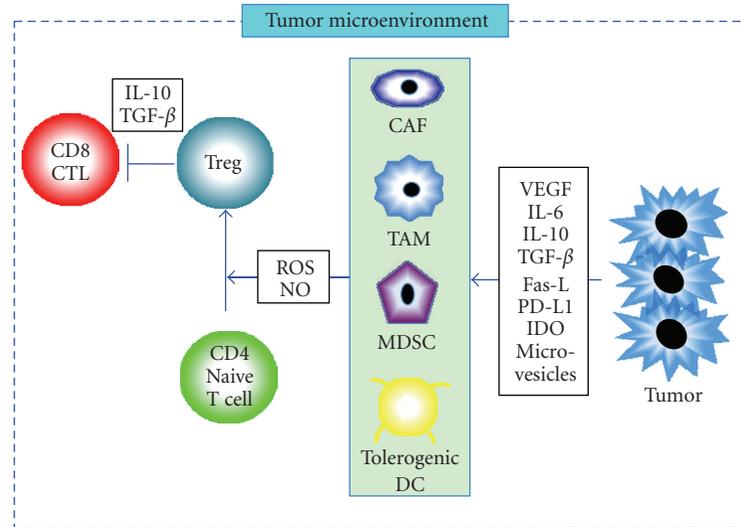


FIGURE 2: Immune suppressive responses at the tumor microenvironment. Tumor cells secrete various factors such as VEGF, IL-6, IL-10, TGF- β , Fas-L, IDO, PD-L1, and microvesicles, all of which promote the accumulation of heterogeneous populations of tumor-associated macrophage (TAM), myeloid-derived suppressor cell (MDSC), or tolerogenic DC. These immunosuppressive cells inhibit antitumor immunity by various mechanisms, including elaboration of reactive oxygen species (ROS) and nitrogen oxide (NO). The tumor microenvironment also promote the accumulation of regulatory T cell (Treg) that suppresses CD8+ CTL function through secretion of IL-10 or TGF- β from Tregs and tumor cells.

and undefined antigen-specific antitumor activities, immunologic cellular monitoring for the fusion vaccines is much more complex. Furthermore, as immune responses induced by DC/tumor fusion vaccines are a balanced mosaic of both immune stimulatory and suppressive responses [92], multiple monitoring assays for the clinical efficacy parameters may be needed to evaluate the antitumor immune responses.

6.1. T-Cell Proliferation. T-cell proliferation assay assesses the number and function at the level of the entire T-cell population in the culture. Therefore, the ability to detect T-cell responses is based on the proliferative potential of the cells in response to antigens. The most commonly used in vitro method for measuring antigen-specific T-cell proliferation is the assessment of T-cell clonal expansion following incubation of T-cells with antigens in the presence of a radio-labeled nucleotide (e.g., [3 H] thymidine) in vitro. CFSE (5-(and-6)-carboxyfluorescein diacetate succinimidyl ester) staining can be also used to directly detect proliferative responses of T-cells [82]. Because CFSE is partitioned equally during cell division [133], this technique can monitor T-cell division and determine the relationship between T-cell division and differentiation in vitro and in vivo. The extensive T-cell proliferation can be demonstrated by the few undivided T-cells left and from proper accumulation of activated T cells, as shown by the increase in T-cell counts correlating with the decrease in CFSE label for each division. The CFSE-based assays are equivalent to traditional measures of antigen-specific T-cell responsiveness and have significant advantages for the ability to gate on a specific population of T-cells and the concomitant measurement of T-cell phenotype [134]. After vaccination, DC/tumor fusion cells can migrate to the T-cell area in the

TABLE 1: Immunologic monitoring.

Inflammatory skin reaction	DTH
T-cell proliferation	[3 H] thymidine uptake CFSE dilution
Cytokine profile	ELISPOT assay Secretion of cytokines Intracellular cytokines
CTL assays	51 Cr-release assays Flow cytometry-based cytotoxicity assays (Caspase-3, Annexin-V)
CTL-associated molecules	Perforin Granzyme B CD107a and b expression in CD8+ T cells CD154 expression in CD4+ T cells
T cell phenotype	Multimer analysis TCR analysis
Immune suppression assays	CD25, FOXP3, IL-10, TGF-beta DTH; delayed type hypersensitivity CFSE; 5-(and-6)-carboxyfluorescein diacetate succinimidyl ester TCR; T-cell receptor

regional lymph nodes and form clusters with CD8+ and CD4+ T cells [34]. Simultaneous recognition of cognate peptides presented by MHC class I and class II molecules on DC/tumor fusion cell is essential in the induction of

efficient CTLs. Therefore, measuring antigen-specific CD8+ and CD4+ T-cell proliferation is essential to evaluate the induction of vaccine-specific immune responses. Although T-cell proliferation assay is usefulness to detect immune responses in vitro, the results are strongly influenced by the in vitro stimulation procedures. Stimulation of naive T cells from healthy donors with DC/tumor fusions in vitro results in potent proliferation of CD4+ and CD8+ T cells [34, 80]. Therefore, to assess DC/tumor fusion vaccines, antigen-specific CD4+ and CD8+ T cells need to be expanded by stimulation with autologous tumor lysates [103]. In addition, the frozen peripheral blood mononuclear cells (PBMCs) obtained before and after vaccination must be processed in the same set of experiments [103, 135, 136]. As T-cell proliferation assay is biologically irrelevant and imprecise for the reasons stated above, this assay may not be emphasized in future studies.

6.2. Cytokine Production. There is a currently great interest in the assay of polyfunctional T cells, secreting multiple cytokines (e.g., secreting IFN- γ and TNF- α rather than either alone), or expressing multiple surface markers. As the release of Th1 cytokines such as IFN- γ and TNF- α is important to determine long-lasting antitumor immunity, a shift to Th1 response by cancer vaccines is essential for therapeutic potential in murine models [36, 37, 67, 77, 137, 138]. Therefore, it is important to test whether cancer vaccines can induce a Th1 response in the tumor-specific T cells, and what impact might this have on the clinical responses. Cytokine production by T cells in response to antigens can be detected in individual T cells by enzyme-linked immunospot (ELISPOT) assay [18–21, 139]. Moreover, production of IFN- γ captured by antibodies bound to T-cell surface can be detected by flow cytometry analysis [96, 140]. The actual state of antigen specific T-cell reactivity directly from peripheral blood T cells can be quantified by IFN- γ ELISPOT assay and flow cytometry analysis [18–21, 141]. As the IFN- γ ELISPOT assay shows highly reproducible results among different laboratories, the ELISPOT may be an ideal candidate for robust monitoring of T-cell activity [18–21, 142]. Coculture of CD4+ and CD8+ T cells from healthy donors with DC/tumor fusions results in high levels of IFN- γ production and low levels of IL-10 production [50, 54, 80, 143]. Therefore, to assess DC/tumor fusion vaccines precisely, T cells obtained before and after vaccination might be directly quantified with stimulation of autologous tumor lysates in vitro [103]. In effective clinical responders, comparable levels of IFN- γ production in response to tumor lysates may be detected in PBMCs obtained before vaccination. A correlation between IFN- γ ELISPOT outcome and effective clinical responses (period free of relapse or survival) has been found in patients treated with cancer vaccines including DC/tumor fusions [103, 135, 136, 144].

6.3. CTL Assays. For immune monitoring of cancer vaccines, T-cell-mediated CTL assays are appealing because measurement of the ability of CTL to kill tumor targets is thought

to be a relevant marker for antitumor activity. It has been assumed that the cytotoxicity has been measured in ^{51}Cr -release assays in vitro. One drawback to the CTL assays is their relative insensitivity. Instead of ^{51}Cr release assays, flow cytometry-based methods have been developed to assess CTL activity [145, 146]. Flow cytometry CTL assays can be predicated on measurement of CTL-induced caspase-3 or annexin-V activation in target cells through fluorescence detection, which are more sensitive to conventional ^{51}Cr release assays [145–147]. These assays show increased sensitivity at early time points after target/effector cell mixing and allow for analysis of target cells in real time at the single-cell level. However, it is unusual to detect antigen-specific killing by T cells directly isolated from the patients vaccinated with DC/tumor fusions even with the use of flow cytometry-based CTL assays [103, 148]. Therefore, there is a need to stimulate and expand the antigen-specific T cells in vitro for several days. These stimulations may distort the phenotype and function of the T-cell populations from tumor state. Moreover, it is difficult to obtain sufficient numbers of viable tumor cells from primary lesion due to the length of culture time and potential contamination of bacteria and fungus [79]. Thus, semiallogeneic targets with shared TAAs and MHC class I molecules are necessary instead of autologous targets. Importantly, a majority of the antigen-specific CD8+ CTLs in peripheral blood may not be tumor reactive due to various mechanisms such as downmodulation of MHC class I molecules on tumors and presence of Tregs at the tumor site. Indeed, cytotoxic activity against autologous targets has been observed in peripheral blood T cells from patients vaccinated with DC/tumor fusions by CTL assays [103, 148], but the clinical responses from early clinical trails in patients with melanoma, glioma, gastric, breast, and renal cancer are muted [103, 130, 134, 135, 142, 143, 148–154]. The defects of the clinical responses may be caused by the immunosuppressive influences derived from the local tumor microenvironment [103]. In addition, therapeutic antitumor immunity depends on highly migratory CTLs capable of trafficking between lymphoid and tumor sites [155]. Therefore, localization of antigen-specific CTLs demonstrated by analysis of biopsy samples from tumor sites may be directly associated with clinical responses [155].

6.4. Tumor-Specific CD8+ and CD4+ T Cells. The population of CD8+ CTLs can destroy tumor cells through effector molecules (e.g., perforin and granzyme B) [156]. Degranulation of CD107a and b is a requisite process of perforin/granzyme B-dependent lytic fashions mediated by responding antigen-specific CTLs. These perforin/granzyme B-dependent lytic fashions require degranulation of CD107a and b in CD8+ CTLs [5]. Therefore, measurement of CD107a and b, perforin, or granzyme B expression by flow cytometric analysis can be combined with intracellular IFN- γ staining to more completely assess the functionality of CD8+ CTLs [83, 87]. Moreover, autologous tumor-induced de novo CD154 expression in CD4+ T cells is highly sensitive for tumor-specific Th cells [157]. The coupling of CD154 expression with multiplexed measurements of

IFN- γ production provides a greater level of detail for the study of tumor-specific CD4+ T-cell responses. Although DC/tumor fusion vaccines have abilities to induce CD107+ IFN- γ + CD8+ T cells and CD154+ IFN- γ + CD4+ T cells upon autologous tumor encounter in vitro [83, 87], it has now been unclear the correlation of the assay with clinical outcome.

6.5. Multimer Assays. Now, it has become possible to analyze antigen-specific CD8+ and CD4+ T cells by flow cytometric analysis using multimeric MHC-peptide complexes, measuring the affinity of the TCR to a given epitope [158]. The MHC-peptide multimer analysis is more sensitive to conventional CTL assays [158]. Although DC/tumor fusion vaccines can induce defined and undefined antigen-specific CD8+ and CD4+ T cells, the multimer analysis can only be used to detect immune responses against defined antigenic epitopes expressing in tumor cells [21]. MHC-peptide multimers stably bind to the TCR exhibiting a certain minimal avidity. Hence, there are principal limitations of the multimeric analysis including the suitability and specificity of multimers and the lack of information about the functionality of multimer-positive T cells [158]. The specific role of the multimer-positive T cells for cancer vaccine efficacy has not yet been well established in the setting of clinical trials. Recent studies suggest that effective cancer vaccines not only stimulate CTL activity, but also sustain long-term memory T cells capable of mounting strong proliferative and functional responses to secondary tumor antigen challenge [159]. Therefore, it is more important to assess whether multimer-positive T cells are effector or effector-memory cells. Moreover, the combined use of multimers and functional assays such as IFN- γ analysis may have provided some insight into the functional activity of these cells. It has been demonstrated that cryopreserved PBMCs from melanoma patients vaccinated with gp100 peptide show that the majority of multimer-positive CD8+ T cells had either a long-term “effector” (CD45RA+ CCR7–) or an “effector-memory” (CD45RA– CCR7–) phenotype [160]. Interestingly, after vaccination, the resected melanoma patients can mount a significant antigen-specific CD8+ T cell immune response with a production of IFN- γ and high proliferation potential [160]. To date, no studies have evaluated the functional activity of multimer-positive T cells in the blood after vaccination with DC/tumor fusions.

6.6. TCR. Only T cells having a TCR specific for a given antigen are triggered by interaction with cancer vaccines. This activation results in the clonal expansion of antigen-specific T cells that can be followed by TCR V β gene usage. Recently, the availability of a large panel of monoclonal antibodies against TCRs, mainly V β epitopes, allows one to study the TCR repertoire by flow cytometry [161]. PCR techniques can also be used to detect a restricted TCR repertoire from small amounts of T cells without biases caused by ex vivo expansions [162]. Although DC/tumor fusion vaccines have resulted in selection and expansion of T-cell clones [87], the generation of antitumor immunity

by CTLs has not correlated with clinical responses. Tumors may evade surveillance of CTLs by distinct mechanisms. Immunogenic tolerance to a particular set of antigens is the absence of an immune response to those antigens, which can be achieved by processes that result in T-cell anergy (antigen-specific unresponsiveness), T-cell unresponsiveness (generalized dysfunction), and T-cell deletion (apoptosis) [163]. Future fusion vaccine studies should be designed to determine whether T-cell dysfunction correlated with clinical outcome.

6.7. Immune Suppression Assays. Although antigen-specific CTLs can be generated and detected in the circulation of vaccinated patients, these do not usually act against the tumor. It has been documented that immune suppressive cells can counteract antitumor immune responses. In tumor microenvironment, there are not only CTLs but also many immune suppressive cells such as CD4+ CD25high+ Foxp3+ Tregs [103, 164], MDSCs [130], TAMs [131], and CAFs [132] (Figure 2). Moreover, tumor cells produce immunosuppressive substances such as transforming growth factor β (TGF- β) [165] vascular endothelial growth factor (VEGF) [166], IL-6 [167], IL-10 [167], soluble Fas ligand (Fas-L) [168], programmed death-1 ligand (PD-L1) [169], indolamine-2,3-dioxygenase (IDO) [170], and microvesicles [171]. Type 1 regulatory T cells (Tr1) expressing CD39 may mediate suppression by IL-10, TGF- β , and adenosine secretion, and whereby accumulation strongly correlates with the cancer progression [172]. The mechanisms that suppress the immune system provide a fundamental reason why cancer vaccines fail to induce consistently robust antitumor immune responses. In DC/tumor fusion vaccines, CD4+ CD25high+ Foxp3+ Tregs were promoted in the presence of the local tumor-related factors in vitro [103]. Moreover, increased CD4+ CD25high+ Foxp3+ Tregs impaired the effector function of CTLs induced by DC/tumor fusion vaccines [103]. Therefore, monitoring of immune suppressive cells in cancer patients vaccinated with DC/tumor fusions is also essential.

7. Conclusion

The development of assays for detecting immune responses associated with clinical outcome has been limited. A variety of assays had been introduced to provide monitoring tools necessary for following changes in the frequency of antigen-specific CTLs and to assess the impact of cancer vaccines on the immune system. As the mechanisms of immune response that cause tumor regression are not simple, the currently available assays may not actually measure a function with direct relevance to how tumors are actually attacked immunologically in cancer patients. A high reproducibility of results among different laboratories leads to the conclusion that cytokine flow cytometry or ELISPOT may be an ideal candidate for robust and reproducible monitoring of T-cell activity in vivo. However, the widely used ELISPOT assay often does not correlate the best with clinical outcome [173]. Therefore, it may be important to use a functional assay like

cytokine flow cytometry or ELISPOT in combination with a quantitative assay like multimers for immune monitoring. Furthermore, it is necessary to understand the immune responses seen in peripheral blood versus the responses at the tumor site. Monitoring of antigen-specific CTLs at the tumor site may be directly associated with clinical responses [155]. However, T cells from lymph nodes and the tumor site itself are not readily available for monitoring purposes in almost all patients. Therefore, the ability to assess the function of antigen-specific T cells from DTH site may serve as an additional strategy to evaluate cancer vaccines [122, 126, 127]. In our opinion, monitoring of multimer-positive CD8+ (effector or effector memory) T cells from the DTH sites or PBMCs with IFN- γ production by flow cytometry may be sensitive markers particularly associated with overall survival. In addition, the DC/tumor fusion vaccine studies should be designed to determine whether T cell dysfunction in the tumor microenvironment correlated with clinical outcome. This information may help us more fully understand the mechanisms of cancer vaccines and its potency to hasten the progress of efficient cancer vaccine strategies into the clinic.

Disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the paper.

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