

Review Article

Regulation of Tumor Immunity by Tumor/Dendritic Cell Fusions

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The goal of cancer vaccines is to induce antitumor immunity that ultimately will reduce tumor burden in tumor environment. Several strategies involving dendritic cells- (DCs)- based vaccine incorporating different tumor-associated antigens to induce antitumor immune responses against tumors have been tested in clinical trials worldwide. Although DCs-based vaccine such as fusions of whole tumor cells and DCs has been proven to be clinically safe and is efficient to enhance antitumor immune responses for inducing effective immune response and for breaking T-cell tolerance to tumor-associated antigens (TAAs), only a limited success has occurred in clinical trials. This paper reviews tumor immune escape and current strategies employed in the field of tumor/DC fusions vaccine aimed at enhancing activation of TAAs-specific cytotoxic T cells in tumor microenvironment.

1. T Lymphocytes and Tumor Immunity

The T-cell receptor (TCR) interaction with complex of peptides and major histocompatibility complex (MHC) molecules is a critical event in T-cell-mediated responses. The proteasomes in tumor cells degrade tumor-associated antigens (TAAs) into short peptides (usually 8–10 amino acids), mostly derived from endogenously synthesized proteins as well as exogenous antigens in the endoplasmic reticulum, and present them to cytotoxic T lymphocytes (CTLs) that express the CD8 coreceptor. Therefore, CD8+ CTLs can directly lyse tumor cells [1, 2]. On the other hand, CD4+ T cells recognize antigenic peptides (10–30 amino acids) associated with MHC class II molecules and mediate their helper functions to induce antigen-specific CTLs through secretion of cytokines such as interferon (IFN)- γ . There are increasing evidences that CD4+ T cells play a more direct role beyond delivery of assistance in the generation of efficient stimulatory immunity [3]. CD4+ T-cell responses can also elicit not only stimulatory but also

suppressive immunity. Now, it is becoming clear that there is an enormous diversity in CD4+ T-helper (Th) cell polarization patterns including Th1, Th2, Th17, and regulatory T cells (Tregs). Th1 cells secrete type I cytokines such as IFN- γ , resulting in the activation of antigen presenting cells (APCs), which can stimulate CTLs [1, 2]. Tumor-specific CD4+ T cells regulate the survival and persistence of CTLs as memory cells [3]. Both CD8+ and Th1 cells secrete IFN- γ , which can further sensitize tumor cells to CTLs by upregulating MHC class I molecules and antigen-processing machinery of APCs. Th2 cells secrete type II cytokines, such as interleukin 4 (IL-4) and IL-10 [1, 2]. Th2 cells can enhance the generation of a humoral immunity, antibody-based antitumor response. The newly identified Th17 cells secrete IL-17, eliciting tissue inflammation implicated in autoimmunity. Finally, Tregs inhibit the development of CTL responses [4]. Tregs are mainly derived from two origins, which are naturally occurring thymus-derived Tregs (nTregs) and adaptive or inducible Tregs (iTregs) [5]. Foxp3 has been considered to be a master regulatory transcription factor

for Tregs [6]. It is becoming clear that Tregs play a pivotal role in the tumor progression and the suppression of tumor immunity [7] (Figure 1).

2. Dendritic Cells (DCs) and Tumor Immunity

Dendritic cells (DCs) are professional APCs and key regulators of T- and B-cell immunity, owing to their superior ability to take up, process, and present TAAs [1, 2, 8]. DCs derive their potency from constitutive and inducible expression of essential costimulatory ligands on the cell surface including B7, ICAM-1, LFA-1, LFA-3, and CD40 [9, 10]. These proteins function in concert to generate a network of secondary signals essential for reinforcing the primary antigen-specific signal in T-cell activation [11, 12]. Therefore, DCs play a pivotal role on the initiation, programming, and regulation of tumor-specific immune responses. Various strategies to deliver TAAs into DCs have been developed to generate potent CTL responses against tumor cells. DCs have been pulsed with synthetic peptides derived from the known TAAs, tumor cell lysates, apoptotic tumor cells, and tumor RNA [13–17]. Another strategy is the use of fusion cells generated by fusing DCs and whole tumor cells [18]. The fusion process facilitates the entry of TAAs, including both known and unidentified, into the endogenous antigen-processing pathway and presents antigenic peptides through MHC class I and II pathways in the context of the potent immune-stimulatory machineries in the DCs [19–22]. These antigen-loaded DCs have already been used as vaccines to improve antitumor immunity [8].

3. Fusions of Tumor Cell and DC

The fusions with whole tumor cell and DC (tumor/DC) by polyethylene glycol (PEG) known as a chemical membrane destabilizing agent [18, 23–25], physical [26–31], or biological means [32, 33] create heterokaryons that express both TAAs and DC-derived costimulatory molecules. Therefore, the fused cells inherit the properties of their parental cells (tumor cell and DC) (Figure 2). For example, the membranes of fused cells are integrated into a single cell whereas the nuclei are remained to be separate, at least in the primary fusions [34]. Such a characteristic structure may make it possible to maintain the functions of both original cells, at least in part, including synthesis of antigens and costimulatory molecules [34].

4. Antigen Processing and Presentation by Tumor/DC Fusions

It has been shown that antigens are processed and presented through two major pathways by DCs. Endogenously synthesized proteins, such as those expressed in viral infections and certain exogenous antigens are processed and presented through the MHC class I-restricted pathway to CD8+ T cells [35, 36]. In contrast, exogenous antigens from the extracellular environment are captured and delivered to the compartments of the endosome/lysosome, where they are

degraded to antigenic peptides by proteases and peptidases, which are complexed with MHC class II molecules and recognized by CD4+ T cells [35, 36]. Importantly, DCs are also capable of processing and presenting exogenous antigens on MHC class I molecules through an endogenous pathway, a phenomenon called antigen cross-presentation [37, 38]. However, the antigen cross-presentation is generally not efficient to induce CTL responses in the absence of carrier proteins or particles [39].

It is now well known how the fusion cells assemble and present the MHC class I- and II-restricted peptide complexes. One possibility is that antigenic peptides are complexed with tumor-derived MHC class I molecules and the complexes are simply transferred and presented by tumor/DC fusions. Moreover, the fusions can efficiently process TAAs from tumors through an endogenous antigen-processing pathway [34]. Therefore, an advantage of the fusions-strategy over DCs pulsed with tumor lysates is that endogenously synthesized antigens have better access to MHC class I pathway [40]. Indeed, tumor/DC fusion vaccines are superior to those involving other methods of DCs loaded with antigenic proteins, peptides, tumor cell lysates, or irradiated tumor cells in animal studies [41]. Moreover, the important advantage of tumor/DC fusions approach is that modifications of tumor cells and DCs are independently possible, which their characters persist after fusion process [22].

5. CTL Induction by Tumor/DC Fusions

Immature DCs take up tumor antigens, mature into IL-12-producing cells, and stimulate Th1 cells in the draining lymph node, resulting in IFN- γ production. These stimulated Th1 cells help during the priming of CD8+ T cells with the capacity for optimal secondary expansion upon re-encounter with antigens. Even in the absence of CD4+ T cells, these memory CD8+ T cells can be rapidly expanded in response to secondary antigens exposure. Expanded CD8+ CTLs can destroy tumor cells through effector molecules such as granzyme B and perforin [42]. Therefore, efficient CTL induction requires the stimulation of both CD4+ and CD8+ T cells. Expression of MHC class I and II molecules, costimulatory molecules (CD80 and CD86), and adhesion molecules (ICAM-1 and LFA-3) on tumor/DC fusions is essential for antigen processing, presentation, and subsequent activation of both CD4+ and CD8+ T cells [25, 43, 44]. In animal models, the fusion cells, like DCs, can also migrate into regional lymph node as early as 18 hours after s.c. injection. Then, the fusion cells localize to the T-cell area in the lymph node and form clusters with CD4+ and CD8+ T cells simultaneously [45].

To dissect the role of antigen-presentation through MHC class I and II pathways by tumor/DC fusions, we created four types of fusions by alternating fusion cell partners: (1) wild-type fusions (WT-FCs), (2) MHC class I knockout fusions (IKO-FCs), (3) MHC class II knockout fusions (IIKO-FCs), and (4) MHC class I and II knockout fusions (I/IKO-FCs) [46]. Immunization of wild-type mice with

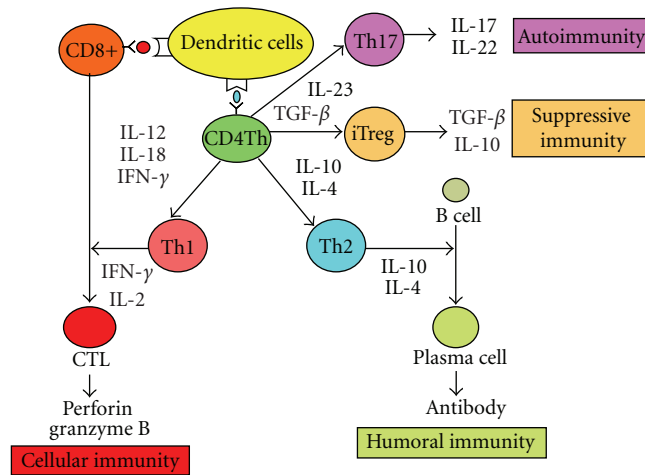


FIGURE 1: The role of helper T cells in tumor immunity. CD4+ T-helper cells play extensive roles and are able to interact with the tumor cell and immune effectors. Th1 cells secrete type I cytokines such as interleukin 2 (IL-2) and IFN- γ , resulting in the activation of DCs, which can stimulate CTLs. Tumor-specific Th1 cells regulate the survival and persistence of CD8+ effector T cells as memory cells. Th2 cells secrete type II cytokines, such as IL-4 and IL-10. Th2 cells can enhance the generation of humoral, antibody-based antitumor responses. Th17 cells secrete IL-17 elicit tissue inflammation implicated in autoimmunity. Inducible CD4+ regulatory T cells (iTreg) exhibit a strong immunosuppressive activity for antitumor immunity.

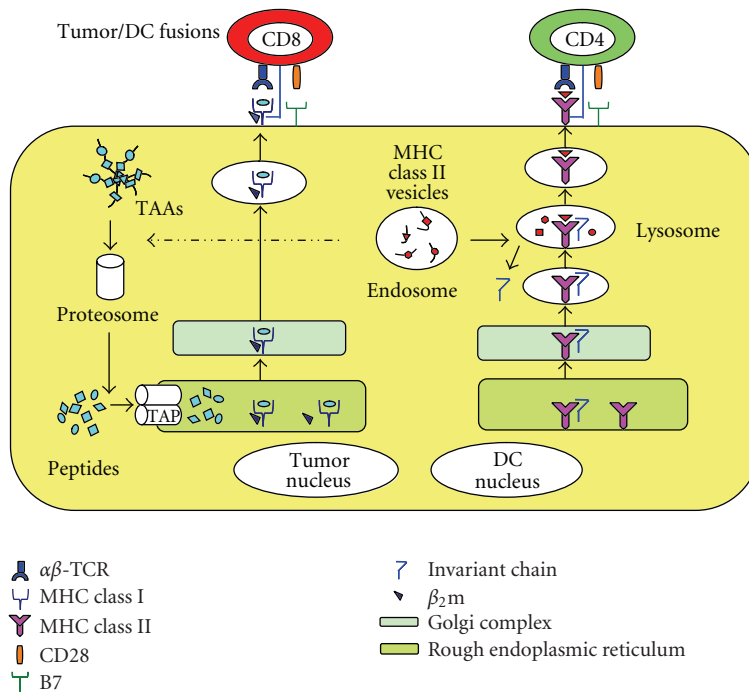


FIGURE 2: Characterization of tumor/DC fusions. Tumor/DC fusions express MHC class I, II, costimulatory molecules and tumor-associated antigens (TAAs). The fusions are able to process tumor-derived peptides and MHC class I peptides derived from DCs. They form MHC class I-peptide complexes, in the endoplasmic reticulum, which are transported to the cell surface and presented to CD8+ T cells. Similarly, the fusions can synthesize MHC class II peptides derived from DC in the endoplasmic reticulum, which are transported to the cytoplasm where MHC class II-peptide complexes are assembled with tumor-derived peptides and presented to CD4+ T cells.

WT-FCs, IKO-FCs, IIKO-FCs, or I/IIKO-FCs provided 100, 91.7, 61.5, and 15.4% protection, respectively, against tumor challenge with MHC class I positive tumor cells. Moreover, IKO-FCs induced slightly decreased tumor prevention and treatment. Importantly, IIKO-FCs abolished IFN- γ production of CD4⁺ and CD8⁺ T cells and CTLs induction. Therefore, antigen presentation through MHC class II is essential for the activation of antigen-specific CD4⁺ T cells and the induction of potent CD8⁺ CTL responses against tumor. Although development of vaccine has been directed toward activation and amplification of CD8⁺ T cells, there is increasing evidence that CD4⁺ T cells play a broader role in antitumor immunity [3]. CD4⁺ T cells contribute to antitumor immunity through diverse mechanisms, in which they are required not only for the maintenance of CD8⁺ CTLs but also for the infiltration of CD8⁺ CTLs at the tumor site [3]. Indeed, adoptive transfer of antigen-specific CD4⁺ T cells controlled tumor growth [46]. Although maximal antitumor immune responses require both MHC class I and II antigen-presentation, MHC class II plays more important roles on the antitumor immunity in cancer vaccines [3, 46]. Therefore, for the design of cancer vaccines, it is essential for activating robust and long-lasting CD4⁺ and CD8⁺ T cell responses in patients with cancer.

6. Tumor/DC Fusions Vaccine

Tumor/DC fusions have been strongly effective in animal studies using melanoma [26, 31, 31, 47–52], colorectal [18, 30, 45, 50, 51, 53–59], breast [60–65], esophageal [66], pancreatic [67, 68], hepatocellular [69–73], lung [74–78], renal cell carcinoma [79], sarcoma [80–85], myeloma [86–93], mastocytoma [94], lymphoma [95], and neuroblastoma [96]. More importantly, in preclinical studies the fusions were also effective to induce CTL responses *in vitro* using colorectal [25, 97–102], gastric [103, 104], pancreatic [105], breast [43, 106–110], laryngeal [111], ovarian [34, 44, 112], lung [113], prostate [114, 115], renal [116, 117], and hepatocellular [118–120] carcinoma, leukemia [121–126], myeloma [127, 128] sarcoma [129, 130], melanoma [29, 131–133], glioma [124], and plasmacytoma [134].

Based on these unique features of tumor/DC fusions with antitumor immunity in murine and preclinical studies, initial Phase I/II clinical trials have been conducted in a variety of tumors (Table 1). Tumor/DC fusions vaccine was first reported in patients with melanoma. Allogeneic DCs were fused with autologous melanoma cells by electrofusion and vaccinated in 16 patients with disseminated melanoma refractory to standard therapy [135, 136]. There were no serious side effects associated with the administration of the vaccine. Seven of the 16 patients responded to the vaccination, one with complete response, one with partial response, and five with stable disease, following to previous rapid progression. Similar results in patients with melanoma were reported from another group using autologous melanoma cells fused to DCs either from healthy donors [137] or the patients [138]. Although Tumor/DC fusions vaccine was also coadministered with rIL-2, efficient antitumor

immunity was not observed in patients with melanoma [139]. Moreover, vaccination with fusions of HLA class I-mismatched DCs from healthy donor and autologous melanoma cells failed to find unequivocal beneficial effects [139]. In addition, in malignant glioma, autologous fusions vaccine produced partial clinical responses in two of six patients [140]. In a similar trial by the same group, a combination of autologous fusions and rIL-12 was administered to patients with malignant glioma, melanoma, breast, gastric, colorectal, and ovarian cancer [23, 24, 141]. Three of 12 patients with malignant glioma achieved a partial response and one patient a minor response [24] but the response to other types of malignant tumors was muted [23]. Another group tested fusions vaccine in 23 patients with metastatic breast and renal cancer [142]. Immunologic and clinical responses were observed in a subset of patients. Two patients with breast cancer exhibited disease regression, including a nearly complete response of a large chest-wall mass. Five patients with renal cell carcinoma and one patient with breast cancer showed stable disease. In a subsequent trial from same group, autologous renal cell carcinoma cells were fused with allogeneic DCs [143]. Although antitumor immune responses were observed in 10/21 evaluable patients, a partial clinical response was demonstrated in two patients and stable disease in eight patients. In patients with renal cell carcinoma, fusions vaccine generated with allogeneic DCs and autologous tumor cells showed immunologic, but not effective clinical responses [138, 144, 145]. Together, only limited therapeutic results were obtained in all these clinical trials.

7. Immunosuppression in Tumor Microenvironment

Tumor/DC fusions aimed for inducing efficient antitumor immunity have provided important proofs of principle in both murine models and preclinical human models. However, immunological responses by DC/tumor fusions vaccine have not been associated with significant clinical responses. A major reason of the diversity is immunosuppressive microenvironment within the tumor. The microenvironment in solid tumors is consisted of tumor cells and stroma cells such as cancer-associated fibroblasts (CAFs), tolerogenic DCs, myeloid-derived suppressor cells (MDSCs), immunosuppressive tumor-associated macrophages (TAMs), and Tregs [66, 146–149] (Figure 3). Tumor cells and CAFs produce immunosuppressive substances such as vascular endothelial growth factor (VEGF) [150], IL-6 [151], IL-10 [151], transforming growth factor- β (TGF- β) [152], soluble Fas ligand (Fas-L) [153], and indolamine-2,3-dioxygenase (IDO) [154]. Tolerogenic DCs express low levels of MHC class I, II, and costimulatory molecules and produce increased levels of TGF- β , all of which are associated with generation of Tregs [155–157]. MDSCs suppress the activation of CD4⁺ and CD8⁺ T cells [158, 159] and also facilitate the generation of tumor-specific Tregs [160, 161]. TAMs promote tumor progression by generation of Tregs [162] and abolish tumor-specific CTLs [163]. As the results, generation of Tregs

TABLE 1: Assessment of clinical trials by tumor/DC fusions-based vaccine.

| Tumor | Tumor/DC Fusions | | Coadministration | Patient Number | Clinical Responses | Ref. | |
|---------------------------|------------------|-----------------|------------------|----------------|--------------------|-------|--|
| | Tumor Cells | Dendritic Cells | | | | | |
| Melanoma | Autologous | Allogeneic | | 16 | 1 (CR) | [135] | |
| | | | | | 1 (PR) | [136] | |
| | | | | | 5 (SD) | | |
| | | | | | 9 (PD) | | |
| | Autologous | Autologous | | 17 | 1 (PR) | [137] | |
| | | | | 1 (SD) | | | |
| | | | | 15 (PD) | | | |
| | Autologous | Allogeneic | | 13 | 8 (SD) | [138] | |
| | | | | | 3 (SD) | | |
| | | | | | 2 (N) | | |
| | Autologous | Autologous | rh IL-12 | 4 | 4 (PD) | [23] | |
| | Autologous | Allogeneic | rh IL-2 | 11 | 1 (SD) | [139] | |
| | | | | | 10 (PD) | | |
| Glioma | Autologous | Autologous | | 8 | 2 (PR) | [23] | |
| | | | | | 1 (SD) | | |
| | | | | | 5 (PD) | | |
| | Autologous | Autologous | rh IL-12 | 12 | 3 (PR) | [24] | |
| | | | | | 2 (MR) | | |
| | | | | | 4 (SD) | | |
| | | | | | 3 (PD) | | |
| Renal cell carcinoma | Autologous | Allogeneic | | 22 | 14 (SD) | [138] | |
| | | | | | 2 (PD) | | |
| | | | | | 3 (OR) | | |
| | | | | | | 3 (N) | |
| | Autologous | Autologous | | 13 | 5 (SD) | [142] | |
| | | | | | 8 (PD) | | |
| Autologous | Allogeneic | | 20 | 2 (PR) | [143] | | |
| | | | | 8 (SD) | | | |
| | | | | 10 (PD) | | | |
| | Allogeneic | Allogeneic | | 8 | 3 (SD) | [144] | |
| | | | | | 5 (PD) | | |
| | Autologous | Allogeneic | | 4 | 1 (SD) | [144] | |
| | | | | | 3 (PD) | | |
| | Autologous | Allogeneic | | 10 | 1 (PR) | [145] | |
| | | | | | 6 (SD) | | |
| | | | | | 3 (PD) | | |
| Breast cancer | Autologous | Autologous | | 10 | 2 (PR) | [142] | |
| | | | | | 1 (SD) | | |
| | | | | | 7 (PD) | | |
| | Autologous | Autologous | rh IL-12 | 2 | 1 (SD) | [24] | |
| | | | | | 1 (PD) | | |
| Gastric/Colorectal cancer | Autologous | Autologous | rh IL-12 | 3 | 1 (SD) | [24] | |
| | | | | | 2 (PD) | | |
| Hepatocellular carcinoma | Autologous | Autologous | | 1 | 1 (PD) | [118] | |
| Ovarian cancer | Autologous | Autologous | rh IL-12 | 3 | 2 (SD) | [24] | |
| | | | | | 1 (PD) | | |

CR: complete response; PR: partial response; MR: mixed response; SD: stable disease; PD: progressive disease.
OR: objective response; N: not evaluated.

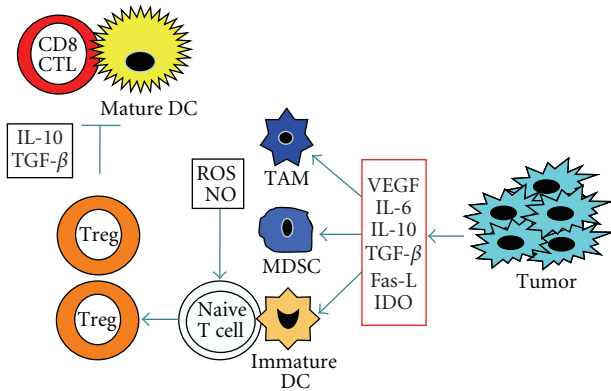


FIGURE 3: Immunosuppression in tumor microenvironment. Tumors secrete various factors such as VEGF, IL-6, IL-10, TGF- β , Fas-L, and IDO, all of which promote the accumulation of heterogeneous populations of tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), or immature DCs. These immunosuppressive cells inhibit antitumor immunity by various mechanisms, including depletion of arginine and elaboration of reactive oxygen species (ROS) and nitrogen oxide (NO). The tumor microenvironment also promotes the accumulation of regulatory T cells (Tregs) that suppress CD8+ CTL function through secretion of IL-10 or TGF- β from Tregs and tumors.

evades the antitumor immunity [164]. Indeed, an increase of Tregs population has been observed in the peripheral blood from patients with advanced cancer [165, 166] and is inversely related to the outcome of several human cancer treatments [167, 168]. Therefore, tumor/DC vaccines that struggle against the tumors with CTLs as well as depletion of Tregs may tip the balance in favor of immunostimulation.

8. Activation or Inactivation of Antitumor Immunity by Tumor/DC Fusions

Progress in antitumor immunotherapy has been aided by advances in the understanding of antigen presentation by DCs and the rules for governing polarization of subsequent immune responses toward CD4+ (Th1/Th2 phenotypes) or CD8+ T cells [2]. Importantly, the immunosuppressive microenvironment in tumors evades CTL responses during their induction and effector phase [165, 166]. Indeed, in cancer patients vaccinated with tumor/DC fusions, soluble factors derived from tumor cells inhibited the induction of CTL responses and promoted the generation of Tregs with immunosuppressive capacities [118]. One way to improve the CTL induction phase may be blockade of the negative soluble factors from tumor/DC fusions. In murine model, tumor-derived TGF- β reduced the efficacy of tumor/DC fusions vaccine via an *in vivo* mechanism [55]. However, the reduction of TGF- β derived from fusions inhibited Tregs generation and enhanced antitumor immunity [66]. Therefore, attention to these immunological bottlenecks may prove critical to fully harness the therapeutic potential of the fusions vaccine. Another approach for blocking the suppressive soluble factors from fusions is the use of

adjuvants. The recognition of microbes by innate immune cells initiates activation of the whole immune system [169]. Toll-like receptors (TLRs) recognize various components of invading pathogens. It has been reported that DCs maturation by microbial products through TLRs is essential for abrogating the activity of Tregs in induction phase of T cells [170]. Moreover, crosspriming by DCs is based on the transfer of proteasome substrates that are transcriptionally upregulated by heat treatment in human tumor cells [171]. Therefore, we have generated mature fusions by fusing DCs stimulated with the TLR agonists and heat-treated tumor cells [100, 101]. The mature fusions had potent APC functions in induction phase of T cells, as demonstrated by (1) upregulation of multiple heat-shock proteins (HSPs), MHC class I and II, TAAs, CD80, CD86, CD83, and IL-12; (2) activation of CD4+ and CD8+ T cells able to produce IFN- γ at higher levels; (3) potent induction of cytotoxic activity specific for TAAs (CEA and MUC1) against tumors. Incorporating heat-treated tumor cells and TLR stimulated-DCs may increase the immunogenicity of tumor/DC fusions in induction of CTL responses. Similar results were also obtained from fusions generated with gastric cancer patients [172]. Immature fusions may stimulate a mixed T cell response characterized by the expansion of both CTL and Treg populations [109]. In addition, tumor/DC fusions activated by TLR agonists, IL-12, and anti-CD3/CD28 preferentially limited the generation of Tregs and promoted expansion of activated CTLs [109, 110]. Therefore, mature fusions have more active to stimulate CTL responses in the immunosuppressive environment in the growing tumor burden (Figure 4). Indeed, in murine models, tumor/DC fusions coadministered with TLR9 (synthetic oligodeoxynucleotides (ODNs) containing specific bacterial unmethylated CpG motifs (CpG ODNs)) and TLR3 agonists (Poly(I:C)) significantly reduced melanoma metastasis through IL-12 production, compared with fusions alone [59, 82]. Moreover, tumor/DC fusions transduced with IL-12 [30, 87, 91, 96], IL-18 [90, 96], GM-CSF [47], IL-4 [88], CD40L [89] genes induced potentially increased therapeutic efficacy.

Another approach designed to improve the efficacy of cancer vaccine is HSP70-based vaccine using tumor/DC fusion technology. The HSP70/peptide complexes (HSP70.PC) derived from tumor/DC fusions were especially different from those derived from tumor cells in enhanced association with immunologic peptides in animal models [173] and human models [172, 174]. HSP70.PC from human fusions induced T cells that expressed higher levels of IFN- γ and exhibited increased levels of killing of tumor cells, compared with those induced by HSP70.PC derived from tumor cells [172, 174]. Moreover, enhanced immunogenicity of HSP70.PC from fusions was associated with improved composition of the vaccine.

9. Combination of Treg Blockade and Tumor/DC Fusions Vaccine

Cancer vaccines must include some strategies to regulate the immunosuppressive cell types and tumor byproducts.

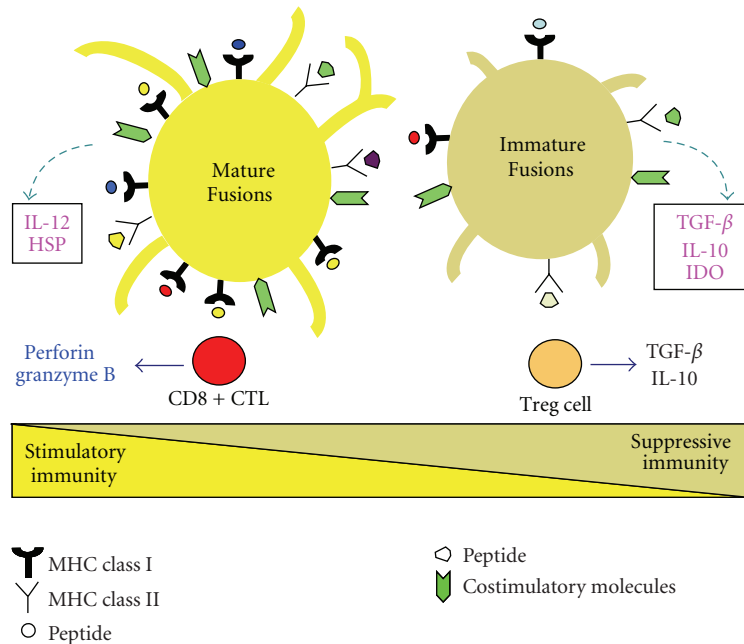


FIGURE 4: Activation or inactivation of T cells by tumor/DC fusions. After acquired antigens in the periphery, tumor/DC fusions migrate to the draining lymph nodes, where they encounter a cognate CD4+ or CD8+ T cells. The mature tumor/DC fusions produce stimulatory factors, such as IL-12 and heat-shock proteins (HSPs), while the immature fusions produce suppressive factors (TGF- β , IL-10, or IDO, etc.). High expression of costimulatory and MHC class I and II molecules by mature fusions is essential to promote survival and proliferative capacity of the activated CD8+ CTLs. Mature fusions induce efficient CD8+ T-cell activation with high production of perforin and granzyme B. On the other hand, immature fusions may induce, at least in part, Tregs. In tumor microenvironment, the consequence of products from tumor cells enhances local suppressive immunity.

Even if tumor/DC fusions were activated by TLRs, Tregs were not a little induced [105, 109, 118]. As Tregs is one of major obstacles for therapeutic cancer vaccines, depletion or blockade of Tregs might enhance rejection of endogenous immune-escaped tumor and improve tumor immunity. In most patients with melanoma (90%), recombinant IL-2-diphtheria toxin fusion protein (ONTAK) treatment resulted in depletion of Tregs and sufficient induction of melanoma-specific CTL responses [175, 176]. Moreover, CTL-associated antigen-4 (CTLA-4) antagonistic antibodies also release a key negative regulatory pathway on T cells and enhance antitumor immunity [177–179]. Other antibodies, such as CD137 (4-1BB) [180], CD40 [181], and programmed death-1 (PD-1) [182] antagonists are currently investigated in various stages of preclinical and clinical development. In tumor/DC fusions approach, it has been reported that the fusions coadministrated with Treg depletion by anti-CD25 antibody enhanced the efficacy of immunotherapy in murine pancreatic models [68]. Therefore, a combination of control of Tregs and concomitant vaccination of mature tumor/DCs fusions may be a more promising approach for the induction of therapeutic antitumor immunity in patients with advanced cancer.

Recently, to overcome negatively regulated pathway by Tregs, a combination therapy of vaccine and chemotherapy has been designed to counteract this immune suppression. For example, when adoptive immunotherapy was combined with nonmyeloablative lymphodepleting chemotherapy, 18

(51%) of 35 treated patients with refractory metastatic melanoma experienced objective clinical responses including three ongoing complete responses and 15 partial responses [183]. This improvement of clinical responses is most likely owing to the elimination of MDSCs and Tregs. Indeed, cytotoxic chemotherapy not only affects the tumor but also depletes MDSCs and Tregs [184]. Postchemotherapy immune system reconstitution may provide a unique opportunity for therapeutic intervention by shaping the repertoire towards responses to tumor antigens [147, 185, 186].

10. Summary

Although immunological responses have been observed in patients with advanced stage of cancer after being vaccinated with DC-based vaccines including tumor/DC fusions, the clinical responses are not as vigorous as in the animal models. Several aspects of cancer vaccines require the reduction of Tregs networks or suppressive tumor-microenvironments that inhibit the function of antitumor immune responses. To date, most of clinical trials have been enrolled patients who are in the advanced stages of cancer, which may have limited the clinical effectiveness because such individuals may not be able to mount an effective immune response. As tumor/DC fusions vaccine has been established as safe in phase I/II trials, the fusions vaccine should be tested in patients with early stage of cancer. Importantly, a combination therapy

of cancer vaccines and other therapies such as conventional chemotherapy should be a more promising approach.

Conflict of Interests

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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