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Potentiation strategies of dendritic cell-based antitumor vaccines: combinational therapy takes the front seat

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Despite recent attempts to take advantage of dendritic cell (DC)-based vaccines for cancer immunotherapy, the results of clinical studies have been disappointing. This is mainly as a result of the diverse immune escape mechanisms used by the tumor together with the insufficient ability of DCs to mount an effective immune response against these mechanisms. In this regard, several approaches have been devised to improve the efficacy of DC-based vaccines. However, the application of each individual approach *per se* might not be sufficient to overwhelm the diverse immune escape mechanisms. In this review, we focus on current strategies for the *ex vivo* potentiation of DC-based vaccines, with an emphasis on combinational therapy methods as a promising alternative for tumor immunotherapy.

Introduction

In recent decades, accumulating evidence has indicated the crucial role of the immune system in the control of tumors and regression of cancers, raising new possibilities for the treatment of cancer through the exploitation of the intrinsic abilities of the immune system. Several observations indicate that the immune system can recognize and even reject tumors. The premise that the immune system could be manipulated for the treatment of cancer has therefore provided a unique platform for cancer immunologists to devise cancer vaccines by harnessing the natural potency of the immune system.

The rationale behind the active immunotherapy of cancer is that tumors take advantage of various immune escape mechanisms to circumvent innate and adaptive immune responses. Active immunotherapy using dendritic cells (DCs) has recently been reported by several studies to 'outsmart' tumors. DCs, as the most potent antigen-presenting cells (APCs), have a pivotal role in determining the fate of the ensuing immune response via the selective activation of specific T lymphocyte subtypes.

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DCs reside in lymphoid and non-lymphoid organs, from which they can be efficiently isolated [1–8]. However, their function is largely influenced by the microenvironment in which they are primed [9–11]. Owing to the existence of immunosuppressive factors, such as transforming growth factor (TGF)-β, interleukin (IL)-10 and vascular endothelial growth factor (VEGF) [12–14], and regulatory T cells (Tregs) in the tumor milieu [15], DC-based immunotherapeutic methods have not been as efficient as first predicted. It has been shown that the tumor microenvironment can shift the cytokine profile secreted by DCs toward a T-helper (Th)-2 pattern [16]. Moreover, DCs residing in cancer tissues usually carry an immature phenotype and are functionally defective in terms of antigen presentation and T cell activation [17,18]. Therefore, current DC-based vaccines have switched to the use of *ex vivo*-generated DCs. These are produced and activated in the absence of a suppressive tumor microenvironment, are more efficient in terms of their antigen presentation and immunogenicity and, therefore, are more able to induce the desired immune response against tumors (Fig. 1).

Following significant advances in the development of DC-based cancer vaccines, the main challenge now is how to optimize and potentiate DC vaccination strategies so that they are more efficient

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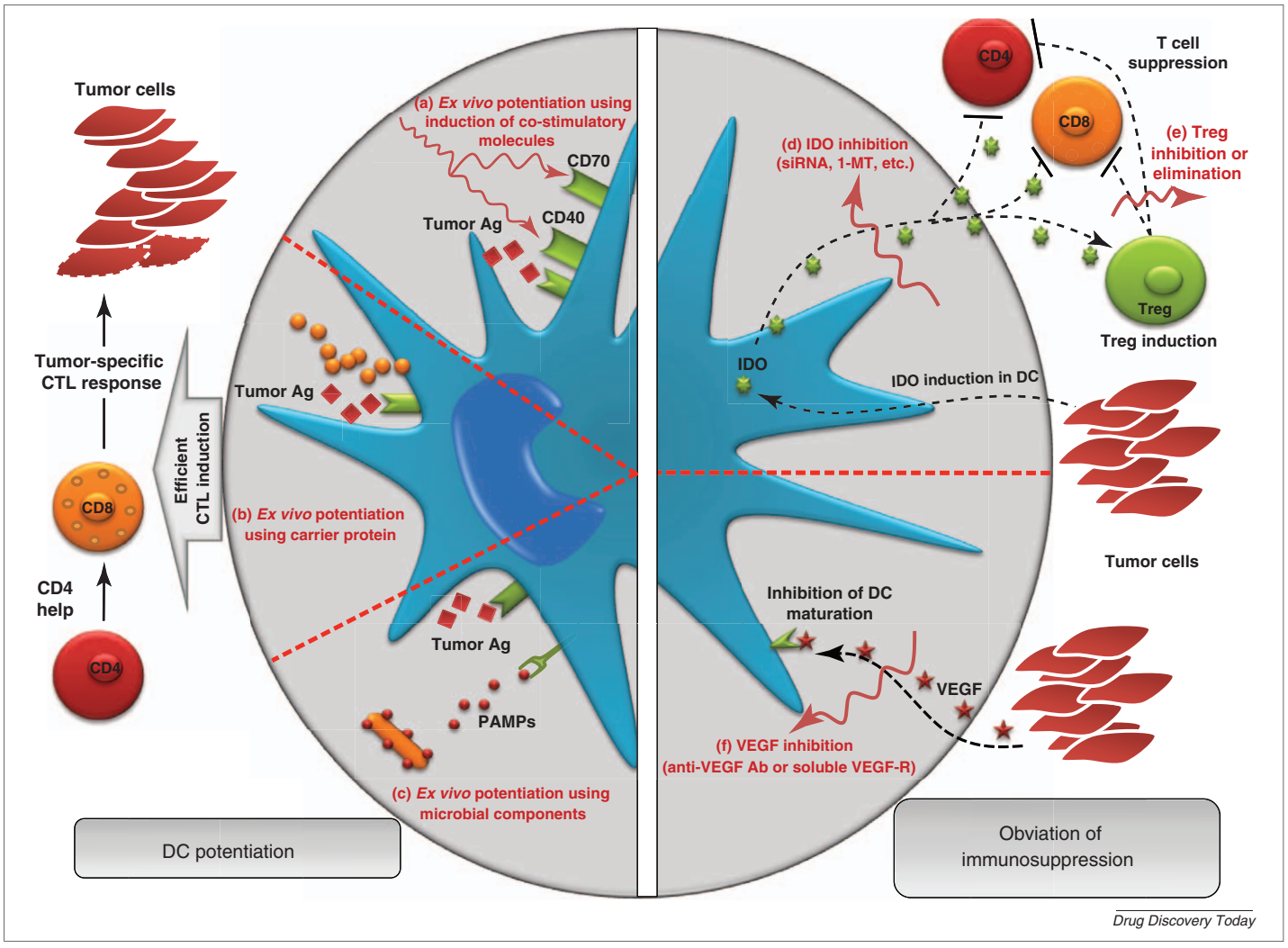

FIGURE 1

Potentiation strategies of DC-based antitumor vaccines. Two general approaches are currently used for the *ex vivo* potentiation of DC-based antitumor vaccines. In the first approach (DC potentiation), modalities such as maturation and activation of DCs by microbial components, trigger co-stimulatory signals and harness the helper effect of carrier proteins, leading to CTL induction and, consequently, tumor lysis **(a–c)**. The second approach (obviation of immunosuppression) 'outsmarts' tumor escape methods through mechanisms including obviation of co-inhibition, inhibition of IDO, Treg elimination and blocking of VEGF activity **(d–f)**. In some settings, a combination of these two approaches is used to harness DC activities that act against tumors.

in the induction of an antitumor immune response. Although several approaches to the *ex vivo* potentiation of DCs have been proposed so far, the application of each individual approach *per se* might not be sufficient to overwhelm the diverse tumor immune escape mechanisms. In this review, we focus on current strategies for the *ex vivo* potentiation of DC-based vaccines, with an emphasis on combinational therapy methods as a promising alternative for tumor immunotherapy (Table 1).

Maturation and activation of DCs by microbial components

Detection by DCs of danger signal, especially microbial components targeting Toll-like receptors (TLRs), induces DC maturation and activation through the ligation of DC receptors; it also induces secretion of type I cytokines and skews the ensuing immune response toward a Th1 response, which favors the antitumor activity of DCs [19,20]. Several studies have exploited this strategy to increase the potency of *ex vivo* generated DCs against tumors by co-culturing them with microbial components.

Recently, more interesting and promising approaches have been devised in which the microbial agent is used to deliver not only the stimulatory signal, but also the tumor antigen to DCs. In an outstanding study, Skoberne and colleagues introduced a killed but metabolically active (KBMA) recombinant *Listeria monocytogenes* as a delivery vector, which facilitated both efficient antigen delivery and full maturation of human DCs along with the secretion of Th1 cytokines. In addition, tumor antigens encoded by *KBMA L. monocytogenes* accessed the endogenous antigen presentation pathway and stimulated antigen-specific $CD8^+$ T cells, leading to the effective priming of tumor-specific $CD8^+$ T cells and lysis of tumor cells [21]. Such an approach provides a promising platform for DC-based vaccination strategies.

DC potentiation by TLR signaling

Knowing that most microbial components exert their stimulatory effects on DCs through TLRs, new approaches have been proposed that take advantage of co-culturing DCs with pathogen-associated molecular patterns (PAMPs) or their analogs to induce DC matura-

TABLE 1

Representative studies using combinational therapy in DC-based cancer vaccines

Approach	Study design	Outcome	Refs
DC activation	Activation of DCs with MPLA and IFN-γ	TH1 polarization of $CD4^+$ T cells; efficient induction of tumor-specific CTL	[22]
	Maturation of DCs with TLR2/4-agonist in combination with IFN-γ	Effective induction of CTL against tumor antigen; increased chemokine secretion by DCs	[23]
	Activation of DCs by TLR3 agonist in combination with depletion or blockade of negative regulatory molecules; for example, B7-H1 on activated DCs	Strong effector of $CD8^+$ T cell responses; protective immunity against newly established tumors	[24]
	Stimulation of DCs via anti-CD40 antibody in combination with IL-12; IL-18-mediated maturation of DCs	Increased expression of IFN-γ and IL-12; induced enhanced $CD8^+$ T-cell proliferation; increased CTL activity; induction of efficient antitumor immunity	[33]
	Electroporation of DCs with a combination of CD40L, CD70 and TLR-4 mRNA	Efficient maturation of DCs; potent IFN-γ-secreting TH1 polarization of $CD4^+$ T cells; increased cytolytic activity of $CD8^+$ T cells	[41]
Obviation of immunosuppression	Dendritic cell vaccine in combination with blockade of VEGF receptor-2 and CTLA-4	Efficient treatment of established and growing tumors, especially larger tumors	[56]
	Combination of CTLA-4 blockade and depletion of Treg cells by antiCD25 with DC-based vaccine	Increased secretion of IFN-γ and enhanced TAA-specific CTL responses; dramatic improvement in tumor-free survival and development of long-lasting immune responses	[57]
	Transfection of DCs with bifunctional siRNA inhibiting IDO and stimulating TLR 7/8	Efficient maturation of DCs in the absence of any external maturation cytokine; efficient activation of T cells	[70]

tion. At present, TLR agonists are widely used as an adjuvant to enhance the immunogenicity of antitumor DC-based vaccines. However, the current trend is toward using TLR agonists in combination with immunostimulatory cytokines, such as interferon (IFN)-γ. As ten Brinke *et al.* demonstrated, the combination of monophosphoryl lipid A (MPLA) with IFN-γ generates more efficient DCs in terms of IL-12 production, polarization of $CD4^+$ T cells toward a Th1 phenotype and induction of superior tumor antigen-specific CTL responses [22]. Promising results were also achieved in a clinical grade study using IFN-γ in combination with a TLR2/4-agonist (FMKp) for DC maturation [23].

TLR agonists also promote potentially negative immunoregulatory mechanisms that attenuate the adjuvant activity of these agonists. In a study by Pulko and colleagues [24], it was shown that polyinosinic:polycytidylic acid (Poly I:C), a TLR3 agonist, consistently upregulated both B7-2 (a positive regulatory molecule) and B7-H1 (a negative regulatory agent) molecules on resident, migratory DCs from spleen and lymph nodes. The authors suggested that the selective blockade of negative regulatory molecules (e.g. B7-H1) in combination with TLR3 agonist is an effective approach to potentiate DCs as a vaccine. Jarnicki *et al.* [25] also reported that TLR agonists induce both IL-12 and IL-10 production and promote not only Th1 cells, but also IL-10-secreting Tregs through the mitogen activated protein kinase (MAPK) p38 signaling pathway. Moreover, the authors showed that selective inhibition of p38 in DCs enhances the efficacy of TLR agonists as vaccine adjuvants and improves the therapeutic efficacy of TLR ligand-activated DC immunotherapy against tumors.

Triggering co-stimulatory signals

It has been well documented that a simple T cell receptor (TCR)–major histocompatibility complex (MHC) interaction is not sufficient for the robust stimulation of antitumor T cell responses and that the co-stimulatory molecules and their ligands on DCs have pivotal roles in triggering antitumor immune responses. Therefore, several investigations have focused on increasing the expression of these molecules on DCs to enhance their antitumor capacity.

CD40-mediated co-stimulation

CD40, expressed on all of APCs and upregulated upon activation, has a crucial role in DC activation [26]. Accordingly, efficient CD40 stimulation on DCs has been considered as a potent candidate for generating efficient antitumor immune responses. However, despite being a potent stimulatory surface molecule, several properties of CD40 confine its clinical development. For instance, agonistic CD40 stimulation via monoclonal Abs led to the suppression of inflammatory responses [27]. Additionally, the CD40 extracellular domain is negatively regulated by several mechanisms [28,29]. Therefore, an innovative set of experiments was recently conducted to target DCs specifically and provide resistance to CD40-regulatory mechanisms, with the aim of extending the pro-stimulatory state of DCs within lymph nodes. In these studies, chimeric molecules consisting of the membrane and cytoplasmic domains of CD40 fused to an extracellular inducible compartment were designed to obtain powerful antitumor immune responses [30]. For example, Yajun *et al.* designed a novel DC vaccination strategy by modifying DCs with a tumor-associated antigen (TAA)-inducible CD40 chimeric receptor. The receptor comprised the CD40 membrane spanning and cytoplasmic domain fused to TAA-specific single chain FV (CD40–scFV). DCs were transfected adenovirally and consequently used to treat breast cancer in a murine model. The modified DCs migrated specifically to TAA-positive tumors, where they were activated

and trafficked to the draining lymph nodes. They consequently induced tumor-specific cellular immunity and suppressed the growth of pre-existing tumors [31,32].

In addition to studies using just a single factor to generate robust immune responses, recent antitumor therapy experiments have shown the advantage of using more than one factor to obtain the best results. For example, Balkow *et al.* applied a combination of different DC maturation stimuli to a murine tumor model. Tumor antigen-loaded DCs were stimulated with anti-CD40, IL-12 and IL-18, and subsequently injected into tumor-bearing mice. Such DCs were able to retard tumor growth efficiently [33].

CD70-mediated co-stimulation

Another co-stimulatory molecule that has received attention from several recent studies is CD70, which is similar to CD40 ligand (CD40L) and is a member of the TNF superfamily of co-stimulatory molecules. CD70 is expressed by activated T and B lymphocytes and some subsets of APCs [34]; it binds to CD27, which is expressed on several cell types, including some subsets of memory T cells. This molecule has a major role in the formation of effector and memory T cell populations [35,36]. Additionally, in some murine studies, it has been shown that CD40 stimulation of DCs leads to CD70 expression on these cells; and that major expansion of $CD8^+$ T cells depends almost entirely on CD70/CD28 expression [37–39]. Interestingly, in a recent study, adoptively transferred CD70-expressing immature DCs were used to prime $CD8^+$ T cells. Co-stimulatory ligand CD70 converted immature DCs from a tolerogenic to an immunogenic state. Such DCs stimulated $CD8^+$ cells to become potent effectors and memory cells with a capacity for a powerful secondary expansion. This raised the possibility of CD70 being a new player in DC–$CD8^+$ T cell interactions aiming at tumor therapy [40].

In a recent study, immature human monocyte-derived DCs were electroporated with a combination of CD40L, CD70 and TLR-4 mRNA. The DCs were matured without the addition of any cytokines and could effectively activate tumor-specific $CD8^+$ T cells *in vitro*. The authors proposed that immature DCs genetically modified to express co-stimulatory molecules could be a potent vaccine for boosting antitumor immune responses [41]. The study also proves the advantage of using several rather than a single potentiating molecule to produce antitumor immune responses.

Harnessing the helper effect of carrier proteins

Exploiting the helper effect of a carrier protein to augment the immunogenicity of tumor antigens is another strategy that increases the immunogenicity and efficacy of DC-based cancer vaccines. In fact, these so-called 'third party antigens' are thought to provide potent $CD4^+$ T-cell help. Timmerman and Levy [42] were the first to show that linkage of an immunogenic protein [e.g. keyhole limpet hemocyanin (KLH)] as a carrier to the tumor antigen increases the potency of DC vaccines. Surprisingly, it has also been shown that not only large immunogenic proteins, but also immunogenic peptides might exert such a helper effect in the context of DC-based tumor vaccines. In this regard, Durantez *et al.* [43] obtained promising results by covalently linking tumor antigens to a complex of four peptides derived from *Staphylococcus epidermidis*. Consistently, in another study [44], it was demonstrated that different arrays of antigens from poorly immunogenic haptens to moderately or highly antigenic proteins can be used to potentiate DC-based cancer vaccines. In addition, these results reinforce the helper effect by enhancing the immune response in a co-pulsing strategy; this is reciprocal and not solely directed to the antigen of interest. Instead, each antigen serves to help the other, a phenomenon termed by the authors as the 'mutual helper effect'. In recent work, it was shown that co-pulsing of DCs with AH-1 peptide and ovalbumin could effectively reduce the tumor size in a murine model of colon carcinoma. Interestingly, a pre-established Th2 anamnestic response against the helper protein considerably weakened its helper capacity (Zarnani *et al.*, unpublished data).

Bacterial toxins have also been used as a carrier protein to potentiate the antitumor efficacy of peptide-pulsed DCs. For example, Fu and colleagues exploited the non-toxic B subunit of *Escherichia coli* heat labile enterotoxin (EtxB) as a carrier to deliver tumor antigen-derived peptides to the antigen presentation machinery of DCs [45]. They showed that pulsing of DCs with conjugates of EtxB-peptide led to efficient MHC class I presentation of antigenic peptides; however, when combined with lipopolysaccharide (LPS)-mediated maturation of DCs, the EtxB–peptide-pulsed DCs induced much stronger peptide-specific $CD8^+$ T cell responses that fully protected naïve mice against B16 melanoma. From this experiment, it can be deduced that these conjugates *per se* serve more as a vector rather than an adjuvant because they did not induce DC maturation in terms of IL-12 production or upregulation of co-stimulatory molecules. Thus, this vaccine setting calls for a complementary method, such as administration of TLR agonists, to be augmented. In this regard, George-Chandy and Eriksson introduced cholera toxin (CT) as an efficient carrier protein that fulfilled both the carrier and adjuvant function for DC vaccines [46–48]. To augment the efficiency of this strategy, these authors and their colleagues [49] devised a combinational therapy using DCs pulsed with CT-tumor antigen conjugates together with intra-tumoral administration of CpG in a mouse model of human papillomavirus (HPV)-induced tumor expressing E7. This vaccination strategy significantly reduced tumor size and completely eradicated the pre-established tumors. Thus, once again, the merit of combinational DC therapy has received positive attention, raising the possibility for the complete eradication of pre-established tumors.

Obviation of immunosuppression

Despite all the recent advances in the field of tumor immunotherapy, only a few therapeutic strategies have been successful in curing several tumors, owing to the suppressive microenvironment imposed by the tumor itself. As previously mentioned, there are several factors, including Tregs, co-inhibitory molecules expressed by tumor cells and some suppressive cytokines that enable tumors to suppress immune mechanisms. Hence, the adoption of approaches focusing on neutralizing tumor-derived suppressive factors could be an effective way of treating cancers. Here, we briefly review some of the factors that enable tumors to either avoid or suppress immune responses, and the experiments conducted to obviate these tumor-specific strategies.

Obviation of co-inhibition

In addition to stimulatory molecules, T cells also express some inhibitory ones, which suppress their activation and proliferation

and are naturally supposed to induce T cell tolerance at different levels. However, expression of ligands of T cell inhibitory molecules on tumor cells can serve as a key factor guaranteeing tumor surveillance. Therefore, it would be tempting to target either the co-inhibitory molecules on T cells or their ligands on tumor cells as an efficient way of conquering the suppressive microenvironment established by tumors.

CTLA-4 blockade

As an immunomodulatory molecule, CTLA-4 has a key role in maintaining peripheral tolerance through suppressing T activation and proliferation. This molecule is expressed on both $CD4^+$ and $CD8^+$ T cells and negatively regulates the stimulatory signals induced by the interaction of CD28 on T cells and B7 on DCs. In addition, CTLA-4 is expressed on $CD4^+CD25^+$ naturally occurring Tregs, inhibiting DC and T cell function [50]. Eliciting T cell responses through CTLA-4 leads to T cell unresponsiveness [51], and its genetic deficiency results in severe auto-immunity in both murine models and humans [52,53].

Therefore, CTLA-4 blockade in combination with DC therapy could be a powerful strategy for the treatment of tumors. In this regard, one study in humans indicated that CTLA-4 blockade together with tumor cell-loaded DC can generate effective antitumor T cell responses [54]. Consistently, in a phase I clinical trial in patients with advanced melanoma, a combination of melanoma peptide-loaded DCs together with a CTLA4-blocking antibody, tremelimumab, induced stronger and more long-lasting antitumor immune responses than did using either agent alone [55]. As previously mentioned, recent studies tend to use more than one potentiating factor when fighting tumors. In one such study, the addition of anti-VEGF receptor-2 to the combination of Ag-loaded DCs along with anti-CTLA-4 antibody proved to be the only effective strategy for inducing the rejection of already-induced established CT26 tumors in a murine model [56]. Furthermore, in a recent approach, a combination of anti-CD25 and anti-CTLA-4 with a DC-based vaccine was used against a murine model of colon carcinoma. The results showed a dramatic improvement in tumor-free survival and development of long-lasting immune responses [57].

PD-1L blockade

Another molecule expressed by some normal and tumor cells is B7-H1 (also called PD-1L), which is a member of the B7 family of co-stimulatory molecules. This molecule primes T cells to produce IL-10 [58,59]. Through ligation with its receptor, B7-H1 also promotes the programmed cell death of effector T cells [60]. Moreover, this molecule is capable of inhibiting T cell growth via ligation of the PD-1 receptor expressed on activated B and T cells [58,61]. B7-H1 is expressed by many cancers, such as several types of ovarian carcinoma. Cancerous cells take advantage of this molecule to induce the programmed cell death of effector T cells, consequently enabling their avoidance of antitumor immune mechanisms [60].

Curiel *et al*. indicated that monocyte-derived dendritic cells (MDCs) upregulate the expression of B7-H1 in the tumor microenvironment, resulting in aberrant T cell immunity and, therefore, tumor escape. Blocking B7-H1 could thus be a way of potentiating MDC-mediated T cell responses. The authors also demonstrated that blockade of B7-H1 increased T cell activation by MDCs and led to the downregulation of T cell-derived IL-10 and upregulation of IFN-γ and IL-12 produced by MDC-activated T cells [62]. Consistently, blockade of B7-H1 in combination with TLR-3 stimulation on DC proved to be an effective way of establishing protective immunity against tumors [24].

Inhibition of indoleamine 2,3-dioxygenase

Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-degrading enzyme that is expressed by a variety of cells and tissues, including macrophages, DCs, cells of the endocrine system, and the placenta and endometrium [63]. IDO was originally considered to be a part of the host innate defense against certain infections by decreasing the available levels of essential amino acid tryptophan; however, in 1999, Mellor and Munn [64] proposed a T-cell suppressing and tolerogenic role for IDO that contributed to resistance to the CTL response. It has also been reported that tumor-draining lymph nodes contain large numbers of IDO-expressing DCs. Such DCs inhibit T-cell proliferation, induce T-cell death and suppress antitumor responses, implying the probable role of IDO-expressing DCs in unresponsiveness to immunotherapy [65]. Even in *ex vivo* generated DC preparations used for immunotherapy, induction of functional IDO has been reported upon *in vitro* maturation with a cytokine cocktail [66–68]. Therefore, it appears plausible to enhance antitumor immune responses by the inhibition of IDO in DCs; Ou and colleagues [69] demonstrated that blocking the activity of IDO with 1-methyltryptophan (1-MT) improved the efficacy of a DC–tumor hybrid vaccine via the induction of a stronger splenic CTL response. In another experiment, Flatekval and Sioud [70] used a small interfering (si)RNA-mediated gene silencing mechanism to inhibit IDO gene expression in DCs. They also devised bifunctional siRNAs having both gene silencing and immunostimulatory activities. The latter function is possible through the chemical modification of siRNAs so that they are able to induce cytokine production through either endosomal TLR 7/8 or cytoplasmic retinoid acid-inducible gene 1 (RIG-1) helicase. The rationale for using such bifunctional siRNAs lies behind the notion that certain siRNA sequences are recognized by endosomal TLR7/8, leading to the expression of IFNs and pro-inflammatory cytokines, particularly IL-12 [71–73]. Moreover, it has been shown that siRNAs bearing 5′-triphosphate inhibit IL-10 gene expression and induce cytokine production. Thus, by transfecting DCs with such 5′-triphosphate anti-IDO siRNA that simultaneously silences gene expression and activates either endosomal TLR or cytoplasmic RIG-1 protein, Flatekval and Sioud induced maturation in monocyte-derived DCs without the addition of any external maturation cytokine, so that these cells were able to activate T cells efficiently. Their study offers a new possibility for DC-based immunotherapy, which places a high value on combinatorial therapy [70].

Treg elimination

One of the main challenges to current cancer immunotherapeutic methods is the existence of Tregs. In case of tumors, these cells have a key role in dampening tumor-specific T cell responses in the tumor microenvironment [74]. Therefore, selective elimination of Tregs has been a major goal in several cancer-directed immunotherapeutic settings, and the results have indicated the rejection of several tumor types following the use of anti-CD25 antibodies [15,57,75].

Depending on their ontogenicity, maturation status and microenvironment, DCs are able to bias naïve T cells toward Th1, Th2 or Treg cells [76,77]. In addition, to use robust strategies to generate potentiating DCs, one needs to take account of the Tregs present in the tumor microenvironment. Indeed, several studies have demonstrated that combining either Treg blockade or depletion along with DC therapy results in a better survival rate and tumor rejection than does using DCs alone. In a recent murine model of glioma, Treg depletion using anti-CD25 antibody *per se* led to a robust protective immunity. However, effective protection upon tumor rechallenge was dependent on the addition of tumor-RNA-loaded DCs to the Treg elimination method, which proves the advantage of using the combination of DC vaccine and Treg depletion over Treg elimination alone [78]. Consistently, in patients with carcinoembryonic antigen (CEA)-expressing malignancies, the efficacy of a combination therapy method using a DC vaccine with Treg depletion was investigated. Tumor Ag-transfected DCs together with a $CD25^{high}$ depleting immunotoxin was used to treat the patients, resulting in the depletion of circulating $CD4^{high}CD25^{high}FoxP3^{+}$ Treg cells along with enhanced anti-CEA T cell responses in vaccinated patients [79].

Blocking VEGF activity

VEGF, known to be secreted by many tumor types, inhibits DC differentiation and maturation in several *in vitro* studies [80]. DC function and number were decreased in several cancers, accompanied by an increase in the plasma level of VEGF [81].

As a consequence, the idea of a blockade or complete clearance of VEGF has become the focus of several investigations aimed at dampening tumor suppressive activities. In one murine model, granulocyte/macrophage colony-stimulating factor (GM-CSF)-secreting tumor cell immunotherapy was used in combination with VEGF blockade to enhance the efficacy of previous methods that had used the former item alone. The combinational approach increased the ratio of the tumor infiltrating effectors to Tregs. This provides evidence for the importance of blocking VEGF in addition to using previous tumor therapies for the treatment of patients with cancer [82]. Consistently, in another experiment, it was shown that although using the anti-CTLA-4 antibody *per se* leads to the survival of tumor recipients, the effective treatment of previously established tumors only occurred when anti-CTLA4 was used in combination with an anti-VEGFR-2 antibody [56].

Briefly, obviating tumor-derived immune suppressive strategies could be overcome by using various methods aimed at dampening, blocking, or completely depleting tumor-serving factors. However, to obtain the best result, one should consider combinational therapies, to make use of more than one weapon to fight against tumors.

Regulation of myeloid-derived suppressor cell function

Myeloid-derived suppressor cells (MDSC) accumulate in the tumor microenvironment in several cancer models [83–85]. These cells are able to suppress cytotoxic T cell responses [86]. MDSC have also been reported to impair DC function and to contribute to tumor development in a model of hepatocellular carcinoma [87]. Hence, several recent investigations have focused on either the functional inhibition or selective elimination of suppressive cells to overcome the insufficiency of immunotherapeutic strategies [88,89]. In one study, the use of formalin-inactivated *Herpes* simplex virus (HSV) as an adjuvant resulted in the activation of DCs and inactivation of MDSCs in tumor immunotherapy [90]. The combination of a DC vaccine with chemotherapy could inhibit the rebound of MDSCs and significantly improve the antitumor effects compared with the control group [91]. By contrast, only a few studies have demonstrated the ability of MDSCs to be converted into immunostimulating APCs, which are able to elicit efficient antitumor responses [92,93]. Collectively, there is a need to take into account the potential effects of MDSCs on the immune system, and in particular on DCs, for most of the strategies using DC vaccines against tumors.

Conclusion

The immune response to foreign antigens encompasses a complex cascade of cell trafficking, antigen uptake, cell–cell crosstalk and antigen presentation, leading to antigen elimination. In fact, tumor cells are able to manipulate delicately components of the immune system at several levels. Therefore, as far as tumor elimination is concerned, potentiation of the immune response against cancer is mandatory.

Owing to the potent antigen presentation and immune stimulating capacity of DCs, they are currently in use as an effective vehicle for introducing tumor antigens to the immune system. Although several approaches for the *ex vivo* potentiation of DCs have been proposed as a robust strategy for improving the efficacy of DC-based vaccines, the application of each individual approach *per se* might not be enough to overwhelm the diverse tumor immune escape mechanisms. The simultaneous use of the immune response potentiation in conjunction with attenuation of tumor immune escape mechanisms appears to be an encouraging approach that has raised new hopes for the complete eradication of pre-established tumors; the concept that 'combinational immunotherapy' refers to.

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