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Dendritic Cell-Based Immunotherapy for Solid Tumors[1,2]

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Abstract

As a treatment for solid tumors, dendritic cell (DC)-based immunotherapy has not been as effective as expected. Here, we review the reasons underlying the limitations of DC-based immunotherapy for solid tumors and ask what can be done to improve immune cell-based cancer therapies. Several reports show that, rather than a lack of immune induction, the limited efficacy of DC-based immunotherapy in cases of renal cell carcinoma (RCC) likely results from inhibition of immune responses by tumor-secreted TGF-β and an increase in the number of regulatory T (Treg) cells in and around the solid tumor. Indeed, unlike DC therapy for solid tumors, cytotoxic T lymphocyte (CTL) responses induced by DC therapy inhibit tumor recurrence after surgery; CTL responses also limit tumor metastasis induced by additional tumor-challenge in RCC tumor-bearing mice. Here, we discuss the mechanisms underlying the poor efficacy of DC-based therapy for solid tumors and stress the need for new and improved DC immunotherapies and/or combination therapies with killer cells to treat resistant solid tumors.

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DC Immunotherapy Effectively Inhibits Tumor Metastasis and/or Recurrence, But Does Not Eradicate Established Solid Tumors

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that drive T cell-mediated immune responses. Vaccination with DCs pulsed with tumor lysates increases therapeutic antitumor immune responses both *in vitro* and *in vivo* [1–3]. DCs capture and process antigens, migrate into lymphoid organs, express lymphocyte costimulatory molecules, and secrete cytokines that initiate immune responses. They also stimulate immunological effector cells (T cells) that express receptors specific for tumor-associated antigens and reduce the number of immune repressors such as $CD4^+CD25^-$ $^+Foxp3^+$ regulatory T (Treg) cells [4–6].

Several reports show that a number of obstacles must be overcome before DC-based immunotherapy can be used as an effective therapy for solid tumors [1,7]. One of the major difficulties with respect to treatment of advanced tumors seems to be that tumor cells suppress the patient's antitumor immune response. Recent reports show that Treg cells, which produce TGF-β, IL-10, and IL-4, play a crucial role in regulating the immune response to self- and/or non-self-antigens [8–11]. Several Treg subsets, such as $CD4^+CD25^+Foxp3^+TGF\text{-}\beta^+$ cells differentiated by tolerogenic (t)DCs, inhibit autoimmune (e.g., rheumatoid arthritis and myocarditis) and inflammatory (e.g., myocardial infarction) [12–15] responses, thereby maintaining immune tolerance in tumor-bearing hosts. In humans with solid cancers, high tumor infiltration by Treg cells and, more importantly, a low effector T (Teff) cell/Treg cell ratio, are associated with a poor outcome [16,17]. Conversely, a high Teff/Treg cell ratio is associated with favorable responses to immunotherapy in both humans and mice

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[18,19]. To date, most studies support the notion that targeting Treg cells, either by depletion or functional modulation, may offer significant therapeutic benefit, particularly in combination with other immune modulatory interventions such as vaccination and checkpoint blockade [20–22].

This review focuses on the mechanisms by which solid tumors escape immune responses and discusses immune cell-based therapy (particularly DC-based immunotherapy) for the treatment of solid tumors.

Recently, a DC vaccination strategy for renal cell carcinoma (RCC), which is based on a tumor cell lysate-DC hybrid, showed therapeutic potential in preclinical and clinical trials [1,23]. Although such trials are promising, several hurdles must be overcome if we are to generate a more effective antitumor response. In a previous study, we developed a murine model of RCC by inoculating mice with mouse renal adenocarcinoma Renca cells. We then examined the efficacy of DC vaccination as a treatment for RCC by testing whether Renca cells induce any of the immunosuppressive effects generally reported in humans with RCC and other solid tumors; such effects might attenuate T cell-mediated antitumor immunity [1]. A DC vaccine would be the best method of inhibiting tumor growth in tumor-bearing mice and would be applicable to humans (cytokine therapies based on IL-12 and IL-2 are not applicable to humans due to toxicity and poor efficacy). Several studies show that, although vaccination with DCs induces a systemic response and can prevent tumor metastasis and recurrence, the response does not affect the solid tumor itself due to its secretion of protective TGF-β. Tumor cells secrete various immunosuppressive factors, including TGF-β [1,24], VEGF [25], and IL-10 [25]. Among these, overexpression of TGF-β is closely associated with a poor prognosis in patients with malignant tumors [26,27]. TGF-β suppresses differentiation of bone marrow (BM) DCs, as well as their capacity to secrete IL-12 [28], present antigens, stimulate tumor-sensitized T lymphocytes, and migrate into tumor-draining lymph nodes [29]. Indeed, we showed previously that high doses of TGF-β inhibit DC migration *in vitro* [1]. However, tumor lysate-pulsed DCs effectively migrated into regional lymph nodes and induced sufficient numbers of functional cytotoxic T lymphocytes (CTLs) in both sc tumor-bearing mice and metastatic tumor-bearing mice. Consequently, we believe that immunity induced by tumor lysate-pulsed DCs may not be restricted to peri-tumor tissue *in vivo*. By contrast, immunohistochemical analyses of sc-implanted tumor masses indicate that the number of Teff cells within the tumor-infiltrating lymphocyte (TIL) population is very low, possibly due to inhibition by tumor-derived TGF-β. These data can be explained by the fact that naïve T cells in sc-implanted tumor masses may be primed to differentiate into Treg ($CD4^+CD25^+Foxp3^+$) cells by tumor-derived TGF-β [30], leading to subsequent inactivation of TILs. Therefore, sc-implanted tumors in mice are resistant to DC vaccination-induced antitumor immune responses. During tumor progression in humans, Treg cells accumulate in tumors and secondary lymphoid organs. Also, chemokines produced by tumor cells or tumor-infiltrating macrophages recruit Treg cells to the tumor bed [30,31].

Although DC vaccination lacks efficacy against sc tumors in mice, it does inhibit further spread of metastatic tumors or tumor recurrence in mice after surgery, indicating that DC vaccination is effective at inducing long-lasting systemic antitumor immunity after surgery [1]. We expect that these results will form an important basis for clinical trials of DC-based immunotherapy under these conditions.

Improved DC Immunotherapy Is Highly Effective at Inhibiting Established Solid Tumors

An immune response is triggered by danger signals, which include microbial products (termed pathogen-associated molecular patterns) and fragments of dying cells; these signals are recognized by the cells that provide innate immunity [32,33]. Of these, DCs are the major link between the innate and adaptive immune responses. Recent reports show that DCs pulsed with tumor lysates *in vitro* and *in vivo* drive increased therapeutic antitumor immune responses after vaccination [1,3,34]. However, several reports show that a number of obstacles must be overcome before DC-based immunotherapy can be used widely to treat tumors [7,35].

In an attempt to overcome such problems, several studies focused on antigen cross-priming using heat shock proteins (HSPs), which are highly conserved and abundantly expressed proteins that have diverse functions [36,37]. Recent studies show that these molecular chaperones interact with APCs; thus their ability to induce antigen-specific CTL and Th1 responses has attracted much attention [38]. In the context of the immune system, HSPs transfer antigenic peptides to $CD8^+$ T cells [38]. During this process, HSP70- or gp96-peptide complexes are internalized by APCs, including DCs, through receptor-mediated endocytosis via CD40, TLR2/4, or scavenger receptor A [39].

Photodynamic therapy (PDT) is an established cancer treatment that uses a combination of light and photosensitizing drugs to damage tumor tissues [40,41]. One of the most important factors induced by PDT is extracellular HSP70 [42,43]; thus we think that *in vitro* exposure of DCs to tumor cell lysates treated with PDT may improve DC immunotherapy against tumors by enhancing their function. Several studies indicate that some HSPs might be suitable [44,45]. Inducible HSPs (i.e., HSP60, HSP70, and HSP90) stimulate DC differentiation and induce expression of several cytokines, including IL-12 [45], thereby increasing their antigen-presenting capacity [46]. Various immune cells, including DCs, macrophages, natural killer (NK) cells, and B lymphocytes, express receptors specific for HSPs [37], including HSP70. Therefore, induction of HSP expression may constitute a 'danger' signal that triggers DC maturation.

It seems likely that PDT-generated tumor lysates contain all of the factors necessary to activate DCs; this may include loading them with antigen and inducing effective antitumor immune responses. In accordance with these findings, HSPs induced by PDT might improve the efficacy of DC vaccines by increasing cross-priming. However, more work needs to be done to fully examine and understand the interaction between tumor antigens and HSPs that is responsible for increasing antitumor responses following PDT-DC vaccination.

Taken together, the data suggest that DCs loaded with PDT tumor lysates are strongly immunogenic and can be used as effective antitumor vaccines [47]. Thus, we expect that PDT-DC vaccination may be developed as an effective immunotherapy for treatment of tumors.

DC Immunotherapy Combined with Cytokine-Induced Killer (CIK) Cells Effectively Suppresses Established Hepatocellular Carcinomas in Mice

Cytokine-induced killer (CIK) cells are a heterogeneous population of *ex vivo*-expanded T lymphocytes with different cellular phenotypes. CIK cells are generated from peripheral blood, BM, or cord blood mononuclear cells upon treatment with a cytokine cocktail (e.g., IFN-γ and IL-2) and an anti-CD3 monoclonal antibody. CIK cells

express markers associated with both T cells and NK cells, including $CD3^+CD56^+$ (NK T), $CD3^+CD56^-$ (typical T), and $CD3^-CD56^+$ (NK). The antitumor activity of CIK cells is mediated mainly by $CD3^+CD56^+$ (NK T) cells, which show NK-like, major histocompatibility complex (MHC)-unrestricted, tumor killing ability [48,49]. Therefore, CIK-based adoptive immunotherapy represents a potential strategy for curing cancer. Indeed, CIK cells exhibit active proliferation and potent antitumor cytotoxicity in the presence of various tumor cells, both *in vitro* and *in vivo* [50].

Although the majority of clinical trials focusing on DC-based immunotherapy have succeeded in generating tumor-specific CTLs in cancer patients, the effects against most solid tumors have been rather disappointing [1,35]. Several mechanisms may account for the limited effectiveness of DC vaccine-induced immune responses to solid tumors. One is that insufficient numbers of $CD8^+$ CTLs are induced in response to DC vaccination alone [51–53]. Alternatively, CTLs generated in this manner may possess suboptimal antitumor efficacy *in vivo*, possibly due to weak activation or inadequate migration to tumor sites [53]. The susceptibility of such cells to host-derived regulatory mechanisms also appears to be a problem.

Preclinical and clinical models in which *ex vivo*-expanded CIK cells have been tested also demonstrate antitumor activity, but only modest therapeutic efficacy; this is due largely to the strategies used by tumors to evade the host immune system [52]. To overcome these problems, we wondered whether a combination of DC vaccination plus CIK cells would induce a stronger therapeutic antitumor effect than administration of DCs or CIK cells alone [54].

CIK cells inhibit proliferation of tumor cells and show tumor cell-specific cytotoxicity. CIK cell-based immunotherapy is associated with a significant increase in the survival rate of cancer patients. Recently, several clinical trials examined combining strategies based on CIK cells with immunization approaches; for example, the combined application of CIK cells and tumor lysate-pulsed DCs improved antitumor toxicity [55]. However, despite several decades of research on CIK cancer vaccines, the clinical effectiveness of immunotherapy remains disappointing.

The goal of cancer immunotherapy is to induce an effective immune response that specifically targets tumor cells. It is well established that activated $CD4^+$ T and $CD8^+$ CTLs are necessary to sustain an antitumor response. Ideally, vaccine-elicited $CD8^+$ T cells should have high avidity and be able to recognize peptide–MHC class I complexes on tumor cells; they should express high levels of granzyme and perforin (molecules essential for cytotoxic activity against cancer cells); they should be able to enter the tumor microenvironment; and they should be able to circumvent immunomodulatory mechanisms in the tumor. At least four components of the immune response are required for this ideal response: the presence of fully-activated DCs; activation of induced IFN-γ-producing $CD4^+$ T helper cells; elimination and/or non-activation of Treg cells; and breakdown of the immunosuppressive tumor microenvironment. DCs generated by *ex vivo* culture of hematopoietic progenitor cells or monocytes with combinations of cytokines have been tested as therapeutic vaccines in cancer patients for more than a decade [56–60].

Our previous report demonstrated that DC vaccination combined with adoptive transfer of CIK cells leads to significant suppression of hepatoma tumor cell growth and improved antitumor responses [54]. These results suggest that a combination of DCs plus CIK cells can increase antitumor activity, indicating a potential for clinical application to cancer patients in the future.

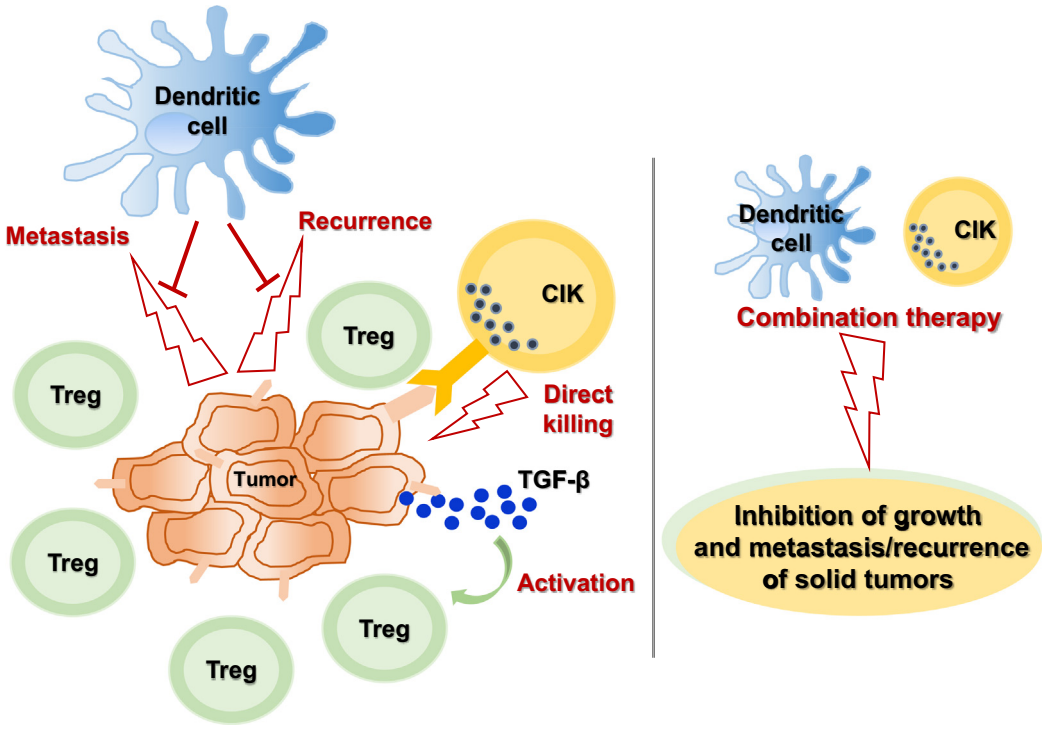

Figure 1. Overview of immune cell-mediated anticancer immunity. DC immunotherapy inhibits the metastasis/recurrence of solid cancers, and killer cells such as CIK cells directly kill tumor cells; a combination of these cell-mediated therapeutics may prove very effective against many difficult-to-treat solid tumors. Treg, T regulatory cell; TGF-β, transforming growth factor-β; CIK, cytokine-induced killer cell.

Conclusions

To overcome the above-mentioned limitations inherent in DC-mediated anticancer immune responses, we suggest the following: 1) depletion and/or inactivation (or non-activation) of Treg cells; 2) development of improved DC-based immunotherapies; and 3) combination therapy based on DCs plus other killer cells (CIK cells, T cells, or NK cells). In particular, we anticipate that DC immunotherapy will inhibit the metastasis/recurrence of solid cancers, and that killer cells such as CIK cells will kill tumor cells; such a combination may prove very effective against many difficult-to-treat solid tumors (Figure 1).

Author contributions

N.-C.J. and D.-S.L. conceived of the presented idea and wrote the manuscript with support from J.-H.L., K.-H.C., and Y.S.K.. All authors discussed the idea and contributed to the final manuscript.

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