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Chapter 9

Cellular Immunotherapy Using Dendritic Cells in Multiple Myeloma: New Concept to Enhance Efficacy

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Additional information is available at the end of the chapter

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1. Introduction

Multiple myeloma (MM) is a clonal B-cell malignancy that is currently incurable with conventional chemotherapy, even if high-dose chemotherapy with autologous or allogeneic hematopoietic stem cell transplantation (HSCT) and the development of novel molecular target agents have resulted in a marked improvement in overall survival [1, 2]. Allogeneic HSCT, which induces a clinically significant immune-mediated allogeneic graft-versus-myeloma (GVM) effect, has provided the framework for the development of immunotherapeutic strategies [3, 4]. To prolong the survival of patients with MM, who are undergoing allogeneic HSCT, a donor lymphocyte infusion can be used successfully as a salvage therapy, which is based on the GVM effect in some cases of MM that relapse after allogeneic HSCT [5, 6]. A clinically significant immune-mediated GVM effect provides the framework for the development of immune-based therapeutic options that use antigen-presenting cells (APCs) with increased potency, such as dendritic cells (DCs), in MM [6].

DCs are the most potent APCs for initiating cellular immune responses through the stimulation of naive T cells. Because of their ability to stimulate T cells, DCs act as links between innate immunity and adaptive immunity in antitumor immune responses [7]. DCs orchestrate a variety of immune responses by stimulating the differentiation of naïve $CD4^+$ T cells into helper T effectors such as Th1, Th2 or Th17 type [8, 9]. Cytokines secreted by DCs at the time of initial T cell stimulation play an important role in the subsequent differentiation of effector T cells. Th1 cells, through interferon-gamma (IFN-γ) production, regulate antigen presentation and immunity against intracellular pathogens [8]. DC-based vaccines have become the most attractive tools for cancer immunotherapy and have been used in more than 20 malignancies; most commonly melanoma, renal cell carcinoma, prostate cancer and colorectal carcinoma

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[10]. Cellular immunotherapy using DCs is emerging as a useful immunotherapeutic modality to treat MM [11]. While antigen-specific cytotoxic T lymphocytes (CTLs) and immune response can be induced by DC vaccination in MM patients, clinical responses so far have been largely unsatisfying to be observed only in a minority of treated patients with MM. Progress in understanding DC biology in cancer patients and the recruitment of suppressive cells of the adaptive and innate immune system in antitumor immunity of cellular immunotherapy is leading to new concept which aims at improved immune and clinical outcomes in MM. New concept is developing to generate novel therapeutic targets that could restore DC capacity to prime T cells and trigger effective anticancer responses in combination with other therapies to offset tumor-induced suppression in MM.

2. Dendritic cell in myeloma immunity

DCs have a potent antigen-specific T cell stimulatory capacity and therefore should be considered to the one of the promising antitumor immunotherapeutic options. In tumor-specific immunity, secreted products or fragments from tumor cells enter into DCs through the endosome and are processed and presented on MHC class molecules of DCs [12]. Processed antigens presented on these molecules of DCs are recognized by $CD4^+$ T helper cells, which not only enhance to the $CD8^+$ T cell response but also facilitate to develop a humoral immune response for surface antigens expressed on the tumor cells. The antigens presented on MHC class I are recognized by $CD8^+$ CTLs, which have a direct cytotoxic effect on tumor cells. Unfortunately, patients with MM have basically dysfunctional DCs that are functionally defective, evidenced by the decreased number of circulating precursors of DCs as well as the impaired T cell stimulatory capacities compared with normal controls [13, 14]. The defective functions of DCs in patients with MM are partially attributed to the production of IL-6 and other tumor-derived factors. DCs in MM patients are a target of tumor-associated suppressive factors, such as IL-10, transforming growth factor- beta (TGF-β), vascular endothelial growth factor (VEGF), and IL-6, resulting in their aberrant functions and impaired development of effector functions in tumor-specific lymphocytes [15]. There were only few patients with MM who responded clinically to vaccination with antigen-loaded autologous DCs. There may be several reasons for this failure from MM patients itself. MM is believed to induce immunoparesis that interferes with DC function and hence affects the effective antitumor immune responses in these patients. They are able to escape immune surveillance by down-regulation of immune markers as well as through the production of immunosuppressive cytokines by the tumor cells or by activation of suppressor cells such as regulatory T cells and myeloid cells. Myeloma cells can produce immuno-inhibitory cytokines, such as IL-10, TGF-β, VEGF, and IL-6, which play major roles in the pathogenesis of MM [15]. In addition, the survival and proliferation of myeloma cells are partially facilitated by impaired endogenous immune surveillance against tumor antigens, including the abrogation of DC function, by constitutive activation of the signal transducer and activator of transcription 3 (STAT3) [13]. Impairment in both humoral and cellular immunity in MM is associated with impaired B cell responses; decreased T cell numbers including $CD4^+$ T cells and impaired CTL responses; and dysfunction

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of natural killer (NK) cells and NKT cells responses [16-19]. In addition, the recruitment and expansion of $CD4^+CD25^+$ regulatory T cells (Tregs) in the suppression of tumor immunity has been reported in MM patients [20, 21]. More recently, the proportion of $CD14^+HLA\text{-}DR^{-/low}$ myeloid-derived suppressor cells (MDSCs) and $CD4^+$ forkhead box P3 $(FoxP3)^+$ Tregs cells was increased in MM patients at diagnosis, resulting in a significant impediment of immune cells related to cancer immunotherapy [22].

3. Current DC vaccination research in MM

Usually, *ex vivo* DCs were generated from circulating blood precursors (i.e. monocytes) or bone marrow progenitor cells and educated them with myeloma-associated antigens prior to vaccination to patients with MM.

3.1. Idiotype-pulsed DCs

Immunoglobulin idiotype (Id) is a tumor-specific antigen that is produced by each B cell tumor clone. Id protein has been used for immunotherapy in patients with MM [23, 24]. Id vaccination could induce immune responses by both antibodies and Id-specific T cells, including $CD4^+$ and $CD8^+$ T cells, through the presentation of Id protein on the surface of professional APCs [24]. Id-specific CTL lines that kill autologous primary myeloma cells *in vitro* have been generated [25, 26]. Autologous DCs that were generated from MM patients have been shown to efficiently endocytose different classes of Id proteins, and autologous Id-specific CTLs that were generated by Id-pulsed DCs were able to recognize and kill autologous primary myeloma cells *in vitro* [25, 26]. Various studies of DC-based Id vaccination in MM have been reported [27-34]. Although Id-specific CTLs and immune responses could be induced in some patients, clinical responses have rarely been observed after vaccination possibly because Id protein is a weak antigen and immature DCs have been used in some studies [27].

3.2. Myeloma-associated antigens-based DC immunotherapy

In general, the production of DC vaccines using whole tumor antigens has become a promising tool for immunotherapy against MM. There are several types of myeloma-associated antigen for loading onto DCs: loading with myeloma lysates [35, 36], loading with dying myeloma cells [37-39], transfection with myeloma-derived RNA [40], pulsing with myeloma-derived heat shock protein (HSP) gp96 [41, 42], and hybridization with myeloma cells [43, 44]. These techniques have the advantage of allowing the presentation of multiple epitopes to MHC on DCs, therefore inducing polyclonal T cell responses from many potentially unknown tumor-associated antigens (TAAs) and reducing the probability of immune escape by a single TAA.

Various myeloma-associated antigens that may induce immune responses from DC-based vaccines have been identified in MM patients. MUC1-specific CTLs that were induced *in vitro* using peptide-pulsed DCs or plasma cell RNA-loaded DCs efficiently killed not only target cells pulsed with the antigenic peptide but also MM cells [40, 45]. DCs transfected with PTD-NY-ESO-1 protein can induce $CD8^+$ cellular antitumor immunity superior to that

achieved with NY-ESO-1 protein alone [46]. Sp17-specific HLA class I-restricted CTLs were successfully generated by DCs that had been loaded with recombinant Sp17 protein and were able to kill autologous tumor cells that expressed Sp17 [47]. The overexpression of hTERT on MM compared to the expression levels in normal cells indicated that this telomerase also could be used as a myeloma-associated antigen. hTERT was capable of triggering antitumor-CTL responses and killing $hTERT^+$ tumor cells [48]. Recently, a report demonstrated that activated T lymphocytes were able to successfully kill myeloma cells after stimulation by DCs loaded with hTERT- and MUC1-derived nonapeptides [49]. DKK1, a novel protein that is not expressed in most normal tissues but is expressed in almost all myeloma cells, may be an important antigenic target for anti-myeloma immunotherapy. DKK1-specific CTLs that were generated by DCs pulsed with DKK1 peptides were specifically lysed by autologous primary myeloma cells and DKK1-positive cell lines [50].

4. New concepts to enhance the efficacy of cellular immunotherapy in MM

4.1. How to enhance the efficacy of DC vaccinations

Because of unsatisfied clinical response of DC vaccination trials in MM, a number of groups have looked at whether the DC vaccination may be more effective if better cytokine combinations are used to enhance DC function, effective tumor antigens are investigated to use, suppressive signal transcriptions are blocked to overcome defective DC function, the interaction with immunosuppressor cells is interrupted to avoid the effect of these suppressor cells, or DC vaccines need to be combined with other therapies.

4.2. The next generation of DCs

To improve DC vaccination, the investigators exploit to the microbial activation signals leading to generate potent DCs with high secretion of cytokines such as IL-12p70, which generate strong tumor-specific Th1 response and helper function for the generation of memory T cells, high production of polarizing signals, which help the generation of high avidity in CTLs that may be resistant to tumor microenvironment, and strong costimulation mediated via several costimulatory molecular pathways [51, 52]. This induces to eliminate Tregs and block tumor microenvironment results in the full activity of elicited CTLs and tumor rejection.

The initial phase of DC-based vaccines involving immature or partially-mature "first-generation" DCs has been reported [53, 54]. However, such DCs express suboptimal levels of costimulatory molecules and constitute weaker immunogens than subsequently implemented mature DCs, the "second-generation" of clinically applied standard DCs (sDCs), which induced by cytokine cocktails containing IL-1β/TNF-α/IL-6/prostaglandin E_2 (PGE_2) [55]. However, to date, sDC vaccines still have some drawbacks, including the mediation of Th2 polarization by increased secretion of the immunosuppressive cytokine IL-10 from DCs and high activity in activating Tregs [56, 57]. Therefore, several investigators, including our group, have tried to develop new generation of potent DC that possess all required features for inducing effective tumor-specific immune responses. We demonstrated the feasibility of

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inducing potent α-type 1-polarizing DCs (αDC1s) by exposing immature DCs to α-type 1-polarizing cytokine cocktail containing IL-1β, TNF-α, IFN-α, IFN-γ, and polyinosinic:polycytidylic acid [poly(I:C)] to generate strong functional CTLs on average 20-fold higher than sDCs [58-62]. Recently, we successfully generated αDC1s from MM patients with high expression of costimulatory molecules, significant production of IL-12p70, and potent generation of myeloma-specific CTLs [37, 38]. Such a novel strategy would provide improved potency of *ex vivo*-generated DCs for cancer immunotherapy.

The other strategy to induce new potent DCs from patients with MM was the use of helper cells to promote type 1-polarization of DCs. Indeed, it has been demonstrated that NK cells play a major immunoregulatory role in the development of protective T cell-mediated immunity against intracellular pathogens and cancers [63]. Such helper activity of NK cells is at least partially mediated by the functional modulation of DCs. This phenomenon depended on the production of IFN-γ and TNF-α from the activated NK cells [63] and was associated with enhanced cross-presentation of tumor antigen and the induction of Th1 and CTL responses [39, 64, 65]. Recent data from our laboratory and other groups has demonstrated that NK-DC interactions promote the subsequent induction of tumor-specific responses in $CD4^+$ and $CD8^+$ T cells, allowing NK cells to act as helper cells in the development of type 1-polarized DCs in responses against cancer [39, 64, 65]. Resting NK cells that are activated in the presence of toll-like receptor (TLR) agonists, IL-2, and IFN-α can induce potent DCs with enhanced IL-12p70 production *in vitro*, generating strong antigen-specific CTLs against myeloma cells [39].

We also found that the selected combinations of TLR agonists synergistically triggered a Th1-polarizing capacity through production of high amounts of IL-12p70 [66]. However, the major limitation of this combination was the decreased ability of these cells to migrate into lymph nodes compared to that of conventional sDCs. When DCs are activated by individual TLR agonists, such as lipopolysaccharide (LPS) or poly(I:C), or by a combination of 2 TLR agonists, all cells mature and produce high levels of bioactive IL-12p70 in early phase of maturation and after subsequent stimulation with T cell-related DC activating signal CD40L. In addition, the phenotyes of these matured DCs were markedly enhanced when a combination of type I and type II IFN was added. These combinations of stimuli also regulated the expression of CD38 and CD74, markers related to the full activation of DCs [67, 68]. We demonstrated that, at the optimal concentration used to stimulate DCs, the combination of 2 TLR agonists with type I and II IFNs can be used to generate fully mature DCs that have high migratory capacity and can maintain IL-12p70-producing capacity. The regulation of CD38 and CD74 in DCs could in turn enhance the migratory activity of DCs in the presence of a combination of 2 TLR agonists and IFNs [69].

Ursolic acid (URC) is isolated from *Uncaria rhynchophylla* and phytochemically classified as a triterpene. Triterpene compounds have been identified as a unique class of natural products possessing diverse biological activities. Recently, we reported that URC activates human DCs in a fashion that favors Th1 polarization via the activation of TLR2- and/or TLR4-dependent IL-12p70 and induces the production of IFN-γ by $CD4^+$ naïve T cells [70]. In addition, combination URC and IFN-γ enhanced the activation of DCs via promotion of IFN-γ-induced Th1

cell polarization that was dependent on the activation of IL-12p70 and independent of TLR4 [71, 72]. The potential of natural products to enhance DC maturation and activation has important implications for the use of DCs as a cancer vaccine.

4.3. New sources of myeloma-associated antigens for DC vaccines

Another important consideration to improve the efficacy of DC vaccination in patients with MM is an effective tumor antigen, instead of using idiotype proteins with a weak antigenicity. The use of whole tumor cells, instead of single antigen, may help to enhance antitumor effects to target multiple tumor variants. It is necessary to use purified, optimized myeloma cells, if possible, as a source of tumor antigens for loading onto DCs to generate potent myeloma-specific CTLs [35]. However, it is not only impractical to obtain sufficient amounts of purified autologous myeloma cells for tumor antigens in the clinical setting from patients with MM and it is also unsuitable for those with a lower tumor burden status. As an alternative source of tumor-relevant antigens, allogeneic tumor cells or established cancer cell lines have been used to overcome the limitation in various tumors [37, 38]. DCs loaded with tumor antigens derived from allogeneic myeloma cells could generate myeloma-specific CTLs against autologous myeloma cells in patients with MM [37, 39]. The success of using an allogeneic myeloma cell line as tumor antigens led to the possibility that allogeneic myeloma cells could also be used as a viable source of tumor antigens in the context of appropriate major MHC alleles to autologous CTLs. In addition, autologous DCs loaded with dying myeloma cells of allogeneic matched monoclonal immunoglobulin subtype showed to generate potent myeloma-specific CTLs against autologous myeloma cells in MM patients [38] These findings suggested that allogeneic myeloma cell lines and allogeneic matched monoclonal immunoglobulin subtype of myeloma were effective tumor antigens capable of inducing functional CTLs against patients' own myeloma cells.

Improved understanding of which specific anticancer agents lead to immunogenic cell death and whether these process can enhance antitumor immunity may facilitate the mechanism how chemotherapy and immunotherapy combination can induce immune responses against cancer. Recently, we have worked to develop strategies that recover dysfunction of DCs caused from loading tumor antigens through treatment of myeloma cells with a combination of the selective JAK/STAT3 inhibitor, JSI-124, and a kind of proteasome inhibitor, bortezomib. We observed that production of inhibitory cytokines, such as IL-10, IL-23, and especially IL-6, which induces DC dysfunction in MM patients, was down-regulated in DCs loaded with dying myeloma tumor cells that induced by these agents. Furthermore, phospho-STAT3 was also down-regulated in the DCs. These DCs displayed a superior ability to induce myeloma-specific responses of CTLs. More recently, we are investigating whether chaetocin could be used to induce dying tumor cells for loading onto DCs to enhance myeloma-specific antitumor responses. We show that anti-myeloma drug-induced dying tumor cells can be used as the source of myeloma antigens to loading onto DCs that could elicit potent anti-myeloma activity of CTLs due to the expression of HSP and cancer testis antigens as a mechanism of immunogenic death of human MM cells.

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4.4. Blocking immunosuppressive activity during the loading of tumor antigens for DC vaccines

The suppressive effects of tumor cells during DC generation have been explained previously by the ability of the tumor microenvironment to suppress DC differentiation [73]. This process can influence STAT3 and ERK phosphorylation, resulting in hyperactivation of STAT3 and ERK, which may be responsible for defective generation of DCs [74]. The immune-mediated antitumor effects of DCs are enhanced by inhibition of the JAK2/STAT3 pathway [75], inhibition of p38 or activation of the MEK/ERK or MAP kinase pathways, and neutralization of IL-6 [76]. Recently, we found that when MM-derived DCs were generated by loading tumor lysates from autologous myeloma cells, these DCs showed lower phenotypic maturation, less T cell stimulatory capacity, less CTL activity, and highly abnormal IL-6 and IL-12 secretion compared to the secretion by unloaded DCs. Moreover, the levels of VEGF, phospho-STAT3, and phospho-ERK1/2 in these DCs were significantly higher than in unloaded DCs. After neutralization of VEGF activity, DC functions, signal transduction, and cytokine production were returned to normal level. Therefore, inhibitory factors and abnormal signaling pathways during maturation with tumor antigens in DCs may be responsible for the defective activity of DCs in MM, and these abnormalities may be overcome by neutralizing the signaling that would lead to a suppressed immune response [77].

5. Combination therapy: New concept to enhance efficacy of DC vaccines

Many factors contribute to the limited clinical efficacy of DC vaccines. The tumor microenvironment contains different kinds of inhibitory cells, such as Tregs and MDSC, and inhibitory molecules, such as IL-10, IL-6, TGF-β, and VEGF, all of which prevent the activation of effector T cells in response to DC responses [16-21, 23, 78, 79]. Although DC vaccines showed effective antitumor effect in experimental *ex vivo* systems, they didn't effectively induce strong immune responses that were enough to kill tumors *in vivo*. Therefore, strategies to improve the efficacy of DC vaccines are to overcome the immune tolerance/suppression induced by these cells, which are involved in the use of a combination of DC vaccine with either stimulatory cytokines or the targeting elimination of inhibitory cells and molecules in tumor microenvironment.

5.1. DC vaccine and cytokine combination

Cytokines, such as GM-CSF or IL-2, known to enhance cell-mediated immune responses may be administered as adjuvants with the vaccines aiming to create an environment where specific immune responses are readily induced [80, 81]. To enhance the efficacy of DC vaccination, Id-pulsed DCs were combined with GM-CSF [80, 82-84], with immunogenic carrier molecules such as KLH [27, 28, 31-33, 82, 85], or cytokine IL-2 [80, 83] to improve the effectiveness of these DC vaccines in patients with MM. Recently, a phase I study was performed in patients with MM using autologous DCs/tumor cells fusion in combination with GM-CSF administration at the day of DC vaccination [86]. The expansion of circulating $CD4^+$ and $CD8^+$ T cells reactive with autologous myeloma cells were detected in 11 of 15 evaluable patients. A majority of

patients with advanced disease demonstrated disease stabilization. In a murine myeloma model, mice were vaccinated with DC-plasmacytoma cell fusions and demonstrated that administration of IL-12 with the vaccine resulted in potentiation of *in vivo* T cell proliferation and cytotoxicity and eradication of established disease [87]. Therefore, the combination of DC vaccine with stimulatory cytokines is a feasible approach to provide a new source of DC-based vaccines for the development of immunotherapy against MM.

5.2. DC vaccine and chemotherapy combination

Chemotherapy can help to reverse the immunosuppression caused by cancers and also further enhance the capacity of DCs to trigger antitumor immunity [88]. Accumulating evidence indicates that conventional chemotherapy as well as radiotherapy selectively eliminates immunosuppressive cells, triggers the activation of DCs, and enhances antigen cross-presentation. Furthermore, specific anticancer agents lead to immunogenic cell death of tumor cells and these processes can enhance antitumor immunity.

Recent studies have shown that chemotherapeutic agents increase the efficacy of active or adoptive antitumor immunotherapies through beneficial immunomodulatory effects [89, 90]. Cyclophosphamide eliminates the activities of tumor-induced suppressor T cells in tumor-bearing hosts [90] and induces the production of immunostimulatory cytokines, such as type I IFN [91]. In addition, low-dose cyclophosphamide has been shown to down-regulate suppressor T cells and to decrease the production of TGF-β and IL-10 while inducing a Th2/Th1 shift in the cytokine profile [92-94]. Low-dose cyclophosphamide may enhance the antitumor efficacy of DC vaccines by increasing the proportion of IFN-γ secreting lymphocytes and suppressing the proportion of $CD4^+CD25^+FoxP3^+$ Tregs in tumor-bearing mice [95]. The result of a clinical trial using allogeneic DC vaccines combined with low-dose cyclophosphamide has revealed that the combination therapy could induce stronger antitumor responses compared to the DC vaccine alone [96]. Recently, we demonstrated that a single administration of low-dose cyclophosphamide before the first DC vaccination showed to augment antitumor effects of DC vaccines to completely eradicate the tumor and to prolong the survival of vaccinated mice [64].

Lenalidomide is a thalidomide analog that has more potent anti-myeloma effects and less adverse effects [97]. Lenalidomide can induce apoptosis of myeloma cells, inhibit the production of cytokines (IL-6, VEGF, and TNF-α) in bone marrow of myeloma patients, and stimulate T cell and NK cell proliferation, cytotoxicity, and cytokine (IL-2, IFN-γ) production [97]. In addition, lenalidomide can inhibit the frequency and function of Tregs, resulting in inhibition of Treg expansion and FoxP3 expression in cancer patients patients [98]. Interestingly, this drug can also induce the activation of APC function, resulting in upregulation of CD40, CD80, and CD86 in chronic lymphocytic leukemia [99]. Therefore, lenalidomide can be used as an immunomodulatory drug in order to enhance immune responses against cancer. Our *in vitro* study showed that lenalidomide enhanced the maturation and function of DCs in the presence of LPS, resulting in synergistic stimulation of DCs to increase phenotype expression, IL-12p70 production, T cell stimulation capacities, and CTL activities against myeloma cells, and to suppress the generation of Tregs. Moreover, our *in vivo* mouse myeloma model showed that

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a treatment combining the lenalidomide with DC vaccination markedly improved antitumor effect by inhibiting immunosuppressor cells, recovering effector cells, and inducing superior polarization of the Th1/Th2 balance in favor of the Th1 response. This immunomodulatory effect may be a crucial component of the enhancer-like properties of lenalidomide in the context of antitumor immunity against MM.

5.3. Chemotherapeutic agent can induce "immunogenic myeloma-cell death" to trigger activation of DCs and to enhance cross-presentation of DCs

Most of chemotherapeutic agents kill tumor cells by the induction of apoptosis. Previously, chemotherapy and immunotherapy have usually been regarded as unrelated therapy in the treatment of cancers because chemotherapy-induced apoptotic cell death has long been considered as non-immunogenic or inducing immune tolerance. Recently, apoptotic cell death when coupled with inflammatory signals, such as HSPs, is clearly known to induce the activation of DCs and triggers the immune response [100]. Some chemotherapeutic agents could induce a type of tumor cell death that activates efficient antitumor immunity, so it is called "immunogenic tumor-cell death". Immunogenic tumor-cell death expresses danger signals on the tumor cell surface or secretes immunostimulatory factors, such as HSPs, calreticulin, high mobility group box 1 protein (HMGB1), and ATP, into the tumor microenvironment, thereby promoting DC maturation and stimulating a powerful T cell immune response [88].

Cyclophosphamide is well known as a potent cytotoxic and lymphoablative drug in conventional and high dosages. However, more recent work highlighted as an immunostimulatory and/or antiangiogenic agent at low dosages, openning up novel indication in the field of cancer immunotherapy. In recent reports, cyclophosphamide administration in tumor-bearing mice induced pre-apoptotic surface translocation of calreticulin on tumor cells [101], which serves as an "eat-me" signal for phagocytes [102] and the release of high-mobility group box1 (HMGB1) protein in the extracellular milieu [101], which constitutes a "danger signal" triggering activation of the DC processing machinery [103]. These events are prerequisites for adequate engulfment of tumor apoptotic material and optimal $CD8^+$ T cell cross-priming by DCs [102, 103].

HSPs are intracellular chaperones for many proteins, but they can also be expressed on the cell surface or even be released under stress conditions [104, 105]. HSP acts as an adjuvant in initiating the activation of DCs or as protein vehicle to facilitate the presentation of antigen peptides to T cells. Spisek et al. [106] reported that uptake of myeloma cells by DCs after tumor cell death induced by bortezomib leads to the induction of antitumor immunity and enhances DC-mediated tumor immune response, indicating the probability mechanism due to the expression of HSP90 on the surface of dying cells, thereby facilitating the activation of DCs in response to dying tumor cells. Our study also found that HSPs released from dying tumor cells, which were induced by a combination of the selective JAK/STAT3 inhibitor JSI-124 and proteasome inhibitor bortezomib, act on tumor cells to recover DC dysfunction and to induce cytokine and chemokine production from DCs, resulting in generation of potent myeloma-specific CTL response against myeloma cells.

5.4. Possible combination DCs and other approaches

In the presence of regulatory and suppressive environment, it is very difficult to elicit or induce effective immune response after DC vaccination in cancer patients. To improve the clinical outcomes, DC vaccines need to be combined, in particular for patients at advanced stages, with other approaches that offset the suppressive tumor environment [107]. It has been known that the specific depletion of $CD4^+CD25^+$ Treg cells by anti-CD25 antibodies increases the efficiency of the anti-tumor immune response of tumor-bearing animals, although the tumors are not completely rejected [108]. An increased number of $CD4^+CD25^+FoxP3^+$ regulatory T cells have been demonstrated in patients with MM [22, 109]. Depletion of Treg may have resulted in improved response to tumor vaccine in animal models and a clinical study. In addition, blocking antibodies or soluble receptors were exploited for the blockade of suppressive cytokines in the tumor microenvironment, such as IL-10 [110], IL-13 [111], TGF-β [112] and VEGF [113]. Such strategies can be used to block immune-inhibitory signals in lymphocytes as illustrated by anti-CTLA-4 [114] and/or anti-PD1 [115] or to block their ligands expressed on tumors.

Another strategy to improve DC vaccination is combination approach with other immune cells, including adoptive T cells or NK cells. In adoptive T-cell transfer, one can seek to modulate the number of regulatory T cells, and transfer a population of activated effector cells. The combination of DC vaccination and adoptive T-cell transfer led to a more robust antitumor response than the use of each treatment modality [116]. These findings illuminate a new potential application for DC vaccination in the *in vivo* stimulation of adoptively transferred T cells. Therefore, combining active and passive immunotherapies in the treatment of MM may enhance the efficacy of tumor vaccine in the future.

6. Future perspectives

Progress in understanding DC biology in MM patients and the recruitment of suppressive cells of the adaptive and innate immune system in antitumor immunity of cellular immunotherapy is leading to new concept which aims at improved immune and clinical outcomes in MM. The new generation of DCs may be a potential vaccine therapy for inducing the rate of tumor responses and prolonging survival of patients with MM. Furthermore, information from studies that combine DC vaccine with other therapies, including chemotherapy, radiation therapy, molecular target agents, other immunotherapy (adaptive T cells or NK cells), or adjuvants will have high impact on enhancing therapeutic immunity in MM by simultaneously enhancing the potency of immune responses and offsetting immunoregulatory pathways.

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