Review Article

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Dendritic Cell-Based Cancer Immunotherapy against Multiple Myeloma: From Bench to Clinic

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Although the introduction of stem cell transplantation and novel agents has improved survival, multiple myeloma (MM) is still difficult to cure. Alternative approaches are clearly needed to prolong the survival of patients with MM. Dendritic cell (DC) therapy is a very promising tool immunologically in MM. We developed a method to generate potent DCs with increased Th1 polarization and migration ability for inducing strong myeloma-specific cytotoxic T lymphocytes. In this review, we discuss how the efficacy of cancer immunotherapy using DCs can be improved in MM.

Key Words: *Multiple myeloma; Dendritic cells; Immunotherapy*

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DENDRITIC CELL-BASED CANCER IMMUNOTHERAPY

Advances in our understanding of cancer immunology have expedited tumor control in the battle against cancer through cancer immunotherapy. At least three approaches for therapeutic intervention can be taken to induce tumor rejection by cytotoxic T lymphocytes (CTLs); these include promoting the antigen presentation of dendritic cells (DCs), enhancing the protective T cell response, and overcoming immunosuppressants in the tumor site.[1] Of these, DCs play the most important role because of their ability to initiate an immune response with ultimate T cell activation, which thus maintains a long-term immune response against the tumor.[2] DC vaccination helps to enforce preexisting antitumor activity or launches a new response through interaction with the T cell repertoire.[1,3] Although the method promises a new horizon for extending survival in cancer patients, it has encountered numerous challenges.

DCs play a sentinel role in tumor control. They have the capacity to activate T cells ($CD4^+$, $CD8^+$), mutually interact with natural killer (NK) cells in tumor immune-surveillance, and directly kill tumor cells.[4] DC-based vaccines are composed of a nontargeted peptide, protein, or nucleic acid captured by DCs *in vivo*, antigen fusion to DC antibody, and *ex vivo*－generated DCs loaded with antigen.[3] However, DCs found in cancer patients are modulated by cancer; hence, their anti-tumor activity is suppressed.[5] The idea to converse with the immune system of cancer patient makes use of *ex vivo*－generated DCs loaded with tumor antigen. In general, this vaccine is composed of activated DCs that are modified by several steps (differentiation, maturation, and antigen uptake) from the monocytes of cancer patients' peripheral blood (Fig. 1).[6] Besides monocyte-derived DCs, plasmacytoid DCs (pDCs) can be used to develop *ex vivo*－generated DCs and have shown promising results for antitumor combat.[7] However, pDCs are less attractive because of their low abundance in peripheral blood. Several combinations of various stimulation factors have been tried to enhance DC maturation with high expression of maturation-related markers, high migration capacity, and increased Th1 cytokine secretion, promoting the generation of CTLs.

MULTIPLE MYELOMA IMMUNITY

Multiple myeloma (MM) is an emerging disease directly associated to clonal plasma cells in the bone marrow microenvironment. The patients suffer from bone lesions, renal insufficiency, anemia, hypercalcemia, and immunodefi-

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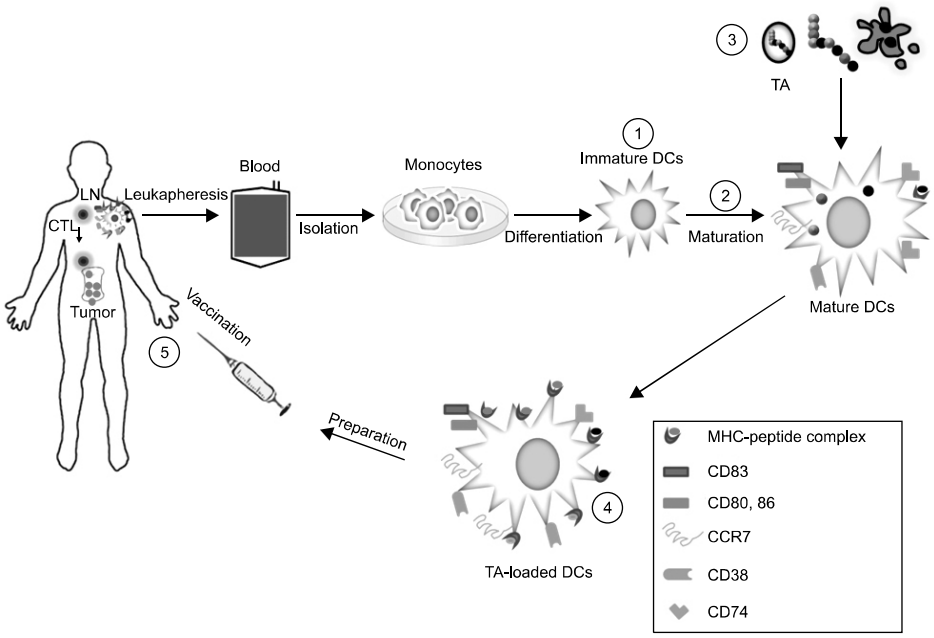

FIG. 1. Critical points for improving cancer immunotherapy using dendritic cells in cancer patients. ① DC engineering (siRNA, DNA transfection). ② Selection of maturation agents (easy preparation, low cost, potent DC induction, and immune enhancement). ③ Tumor antigen modulation (enhance tumor specificity, easy preparation, broad spectrum, easy delivery, increase cross-presentation, reduce immune suppression). ④ DC function enhancement (increase Th1 polarization, reduce regulatory T cell and myeloid-derived suppressor cell activity). ⑤ Vaccine efficacy (increase lymph node homing, modulate tumor environment). LN: lymph node, CTL: cytotoxic T lymphocyte, DCs: dendritic cells, TA: tumor antigen.

ciency.[8] The relationship between MM cells and the cancer niche helps to maintain cancer development. Numerous molecules are released, such as transforming growth factor-β (TGF-β), IL-10, IL-6, vascular endothelial growth factor (VEGF), Muc-1, Fas ligand (FasL), B7-H1, cyclooxygenase-2 (Cox-2), and matrix metalloproteinases (MMPs), that influence immune cells and impose serious challenges in antitumor combat.[9] TGF-β is abundantly expressed by MM cells. The cytokine blocks phosphorylation of STAT3 and STAT5, and hence inhibits T cell proliferation and T cell response to IL-2, thus favoring the development of regulatory T lymphocytes. In addition, TGF-β and IL-10 induce functionally defective DCs in MM patients. IL-6 inhibits Th1 polarization by direct mediation on T cells and promotes Th2 differentiation by inducing IL-4 secretion of $CD4^+$ T cells. On the other hand, IL-6 secreted from MM cells has a negative effect on DC function. VEGF induces T cell exhaustion and DC dysfunction. MUC-1 is aberrantly expressed in MM cells. It contributes to promoting MM cell growth and survival, inducing DC functional defects, and suppressing T cell activation. Expression of FasL and B7-H1 on the MM cell surface promotes apoptosis of tumor-specific T cells. Overexpression of Cox-2 in MM cells affects the balance of IL-10 and IL-12 in favor of IL-10 production, which reduces overall survival in patients. MMP is a member of the proteinase family, which can degrade extracellular matrix. Similar to other mentioned factors, MMP is reported to be involved in bone destruction in patients and in the activation of TGF-β.[9]

DENDRITIC CELL-BASED IMMUNOTHERAPY AGAINST MULTIPLE MYELOMA

1. Idiotype-pulsed DCs

Idiotype (Id) protein secreted by MM cells presents in processed form on the MM cell surface and is considered a tumor-specific antigen.[10] Vaccination of Id-pulsed DCs not only promotes activation of Id-specific CTLs in the immunity repertoire, which can recognize and lyse autologous primary myeloma cells through the perforin-mediated pathway or through both pore-forming perforin and FasL-Fas interaction, but also recruits the humoral immune response.[11] In addition, Id-specific $CD4^+$ Th1 cells can directly induce tumor apoptosis by FasL-Fas interaction and indirectly inhibit tumor growth with secretion of IFN-γ. On the other hand, Id-specific $CD4^+$ Th2 cells promote tumor protection, which is in contrast with a previous publication.[12] Even though Id-pulsed DCs have been used in clinical trials, the response after vaccination was disappointing because Id protein is a weak antigen that generates monoclonal proliferation of T cells, and Id-specific T lymphocytes are anergic in case of excess soluble Id protein in MM patients.[13]

2. MM-associated antigen-loaded DCs

Several MM-associated antigens have been discovered, such as polymorphic epithelial mucin (MUC1), cancer testis antigen (CTA), sperm protein 17, MAGE-1, MAGE-3, MAGE-C2, MAGE-C1, MAGE-A1, MAGE-A3, PRAME,

NY-ESO-1, SSX1, SSX4, SSX5, SSX2, BAGE, ADAM2, LIPI, Dickkof-1, hTERT, CD138, XBP1, CS1, WT1, survivin, and BCMA.[14-24] MUC1 is a mucin molecule, a kind of high-molecular-weight glycoprotein with varied glycosylation among different normal and malignant cells. It is expressed on MM cells and secreted into the patient's serum. DCs pulsed with MUC1 and hTERT nonapeptides show a similar cytotoxicity induction capacity as do DCs loaded with apoptotic bodies.[25] CTAs are frequently expressed on malignant MM cells. However, some of the CTAs show tumor-specific CTLs, such as the MAGE family (MAGE-A1, MAGE-C1, MAGE3) and NY-ESO-1.[15,20,26-28] Addition of a protein transduction domain (PTD) to tumor antigen can favor entrance of the antigen into the cytoplasm, presentation of the antigen on HLA class I, and hence an enhanced tumor-specific CTL response.[27] Dickkopf-1 (DKK1) possesses a restricted expression on placenta and mesenchymal stem cells. RT-PCR results have shown expression in MM cell lines and MM patients. Use of DKK1 to generate DC vaccine show a good CTL response to U266, IM-9 cell lines, and MM cells from patients.[18] DCs pulsed with CS1 peptide show an ability to enlarge effector memory and activate CTLs and the tumor-specific CTL population.[23] To overcome the limitation of the specificity of CTLs against a single peptide, a cocktail of several peptides has been used to pulse onto DCs. The results showed that the vaccine with a peptide cocktail of CD138, XBP1, and CS1 displays an enhanced specific T cell response and initiates a broad-spectrum immune response against MM cells and other plasma disorders.[21,22] Transfection of DCs with mRNA of MAGE3, survivin, and BCMA promotes a tumor-specific CTL response.[28] In general, tumor-associated antigen-pulsed DCs promise a potential vaccine for MM treatment.

3. Whole tumor antigen-loaded DCs

Cancer immunoediting permanently allows a tumor to escape antitumor immunity. Therefore, the tumor can easily be resistant against a given tumor-antigen-specific CTL. For this reason, the use of whole tumor antigen-loaded DCs is considered a promising tool. First, tumor-lysate-pulsed DCs were investigated and were shown to be an effective and safe vaccine.[29] Heat shock protein (Hsp) represents a fingerprint of a tumor and promotes cross-presentation of MHC class I-restricted epitopes. Myeloma-derived Hsp gp96 pulsed-DCs are a safe vaccine that generates myeloma-specific CTLs that can lyse MM cells but do not display any cytotoxic activity to normal cells.[17] Using a pool of heterogeneous MM cell line Hsp as a tumor antigen to load onto DCs induces good protection in mice against tumor development.[30] Alternatively, DCs can be pulsed with apoptotic bodies,[31,32] transfected with tumor-derived RNA,[33] or fused with live MM cells.[34] DC vaccine in this category induces a broad repertoire of CTLs. In comparison with Id-pulsed DCs, tumor lysate-pulsed DCs are more effective for lysing autologous MM cells.[35] The myeloma cell line Hsp is a tumor antigen complexing with Hsp. Hsp chaperone induces DC maturation, promotes cross-presentation of DCs, and skews the T cell response to Th1 polarization.[36] DC/tumor fusion vaccine can elicit both helper T cells and a CTL response through the ability of DCs to present both antigen that has been taken up and newly synthesized antigen inside hybrid cells. The vaccine was shown to be a feasible, well-tolerated, and good antitumor response in a phase 1 clinical study in MM patients.[34] Currently, potent DCs loaded with dying MM cells are being used by our group in phase I/IIa clinical trials in patients with relapsed or refractory MM.

IMPROVEMENTS IN DC-BASED CANCER IMMUNOTHERAPY

1. Type 1-polarized DCs

The onset of DC immunotherapy started with autologous immature DCs as the first generation of DC vaccine. The results showed a good impact on tumor regression and increase in patient survival.[37] However, the weak and short-term response of immature DCs gave rise to second-generation DC vaccines with mature DCs.[38] In the early days of these vaccines, the conventional PGE_2-DCs, called the "gold standard DCs," were considered the most powerful candidate for vaccine development and were shown to favor regulatory T cell attraction.[39,40] Scientists then started to develop several third-generation DC vaccines by applying cytokine cocktails for DC maturation with the aim of generating stronger Th1 induction and higher migration capacity than the second-generation DC vaccine.

The most impressive DC vaccine was the alpha-type 1-polarized DCs, which gave an excellent immune response in several cancers.[31,41-45] However, the number of cytokines, including TLR agonist, used in the maturation cocktail imposed a high cost and the alpha-type 1-polarized DCs showed a lower migration capacity than conventional PGE_2-DCs. Therefore, a reduction in the number of cytokines, a switch to a cheaper maturation agent with the same activity as DCs, or an improvement in certain characteristics of the DCs, such as DC migration, IL-12p70 production, and Th1 polarization, is necessary to generate a better vaccine candidate.[46-51]

Our group has succeeded in generating potent DCs with a reduced number of cytokines. The DCs generated with TLR3 and TLR4 agonist in synergy with IFN-α and IFN-γ were fully matured with high production of IL-12p70 and good migration capacity. In the presence of IFN-α and IFN-γ, the TLR agonists synergistically up-regulate the expression of CD38 and CCR7 and down-regulate CD74 expression. Single addition of IFN-α or IFN-γ to TLR agonists enhances DC migration capacity and the greatest result was obtained in the case of addition of both IFN-α and IFN-γ to TLR agonists.[51] Another maturation cocktail included bacterial flagellin and *Vibrio vulnificus* FlaB (v-FlaB) in combination with IFN-α and TNF-α.[49] v-FlaB could act in synergy with IFN-α and TNF-α for obtaining the highest level of IL-12p70. In addition, the presence of

v-FlaB enhances migration of IFN-α/TNF-α DCs. Compared to other DCs, these DCs have high capacity to induce Th1 polarization (high level of IFN-γ and low level of IL-13 with a high level of expression of the Th1-attracting chemokine IP-10). These DCs can generate antigen-specific IFN-γ-secreting cells with 6-fold higher generation than with conventional PGE_2 DCs. Furthermore, CTLs generated from v-FlaB/IFN-α/TNF-α DCs have great capacity to migrate to the tumor site.

Our group has also investigated the effect of natural products on DC maturation and Th1 polarization. *Uncaria rhynchophylla* possesses two active components, uncarinic acid C and ursolic acid, that induce potent mature DCs. Uncarinic acid C can modulate DC functions that favor a Th1 response. To get more effective DCs, the addition of IFN-γ to uncarinic acid C augmented CCR7 expression of DCs, IL-12p70 production from DCs, and secretion of IFN-γ by CTL cells.[46] Ursolic acid induces IL-12p70 production on DCs in a dose-dependent manner. Use of anti-TLR2 and anti-TLR4 antibodies proved that ursolic acid can generate mature DCs through interaction with TLR2 and/or TLR4.[47] Cryptomerione is classified as a terpene compound and is present in *Cryptomeria japonica*. The presence of Cryptomerione helps to increase the IL-12p70 level and reduce the IL-10 level from cholera toxin-pulsed DCs and to promote DC migration.[48]

Furthermore, based on the cross-talk among DCs, NK cells, and $CD8^+$ T lymphocytes, several DC vaccines were generated through interaction with these helper cells in combination with cytokines and TLR agonists.[52-54] In fact, DCs help NK cells to exert tumoricidal activity and, in turn, NK cells activate DCs to induce maturation and cytokine secretion toward Th1 polarization. DC and NK cell interaction gives rise to mutual activation and cytokine production of both cells. Induction of DC maturation requires 2 signals of helper and effector NK cells. The helper signal of both cells is required by direct contact between DCs and NK cells.[55] Co-culturing of immature DCs with resting or activated NK cells showed that DC maturation was promoted by resting NK cells and dependent on stimulation condition.[54]

2. Tumor antigens to load onto DCs

A good tumor antigen candidate should present a broad tumor-specific fingerprint, the possibility to form MHC class I-peptide complex, ease of preparation, and reduction of immune suppression. As a starting point, Id-proteins were used as a tumor antigen to pulse onto DCs and gave a good immune response in MM. However, a high level of Id-protein in a patient's serum depletes the response. This reduced response led to the application of tumor-associated antigens (TAAs). Most TAAs are also expressed on normal cells at a lower level, which imposes a risk of autoimmune reaction in cancer patients. Fortunately, several investigations have shown that the use of TAAs is feasible and safe for cancer patients. In general, extracellular protein uptake by DCs gives rise to Th2 polarization and the uptake was poor. As a consequence, TAAs were used in peptide sequence form such that the vaccine encounters a limited anti-tumor CTL repertoire associated with certain peptide epitopes. To overcome the problem, a cocktail of peptides, whole tumor cells, and their derivatives were used instead of a single peptide to pulse onto DCs, which activate a broad tumor-specific CTL repertoire.[56]

Whole tumor cell lysate can be prepared through a freezing-thawing procedure, apoptotic bodies from ultraviolet B irradiation (UVB), tumor-derived RNA, tumor-derived Hsp, or live tumor fused with DCs. Tumor lysate of total mononuclear cells obtained from bone marrow can induce an autoimmune reaction related to healthy cells and reduce the capacity of antigen uptake by DCs. We showed that a high concentration of tumor lysates can suppress DC function and that purified $CD138^+$ cell lysate-pulsed DCs can induce a higher CTL response than total cell lysate-pulsed DCs. In addition, DCs pulsed with an optimal concentration of purified tumor lysate could induce a potent myeloma-specific CTL response.[29]

In the clinical setting, it is very difficult to get enough malignant MM cells for DC vaccine preparation. Our group investigated the possibility of DCs pulsed with allogeneic myeloma cells, prepared from an allogeneic MM cell line or allogeneic primary MM cells.[31,42] Interestingly, these DCs could generate a potent myeloma-specific CTL response to the patient's primary MM cells. The results opened the possibility for use of allogeneic tumor antigens for a DC-based vaccine against MM.

Tumor cells express several immune-suppressive molecules that edit anti-tumor immunity; whole cell tumor preparations can contain these cytokines that negatively influence the activity of DCs. VEGF, an angiogenesis agent, is one example of an immune-suppressive molecule that induces DC defects with high IL-6 and IL-10 production and the suppression of IL-12 secretion. Use of anti-VEGF antibody that blocks STAT3 and ERK phosphorylation right after tumor antigen pulsing helps to recover DC function through activation of the NF-κB pathway.[57] In addition, the idea was proposed to prepare a kind of MM antigen that can inhibit Janus-activated kinase 2/signal transducers and activators of transcription 3 (JAK2/STAT3) signaling such as JSI-124 (cucurbitacin-I), which can activate immature DCs and promote the differentiation of mature DCs. Also, bortezomib is a proteasome inhibitor and induces tumor cell death with exposition of Hsp90, which induces DC maturation and enhances cross-presentation to increase the CTL response.[58] A combination of JSI-124 and bortezomib in the pretreatment of MM cells following UVB irradiation was proved to induce strong immunogenic cell death with high expression of Hsp90. Dying tumor prepared by treatment of JSI-124 and bortezomib loading onto DCs showed a decrease in inhibitory cytokines such as IL-6, IL-10, and IL-23 with a good capacity to induce a tumor-specific CTL response.[32]

3. Regulation of the tumor suppressive microenvironment

Immunomodulatory drugs (IMiDs), such as thalidomide, lenalidomide, and pomalidomide, are currently prescribed for MM patients and can reverse the immunosuppressive effect by down-regulation of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). Lenalidomide presents anti-angiogenic activity and anti-tumor immunity that enhances T cell expansion with Th1 polarization by inhibition of Treg development and PD-1 expression, by activation of NK cells and T cells, and by suppression of inhibitory factors, thereby enhancing tumor-specific immune responses.[59,60] An investigation of the effect of these drugs on DCs showed that pretreatment of DCs with IMiDs could induce $CD8^+$ T cell proliferation with high levels of IFN-γ and perforin. These drugs were considered an adjuvant for DC vaccination.[61] In addition, IMiDs showed a synergistic effect with TriMix (an mRNA cocktail encoding for TLR4, CD40L, and CD70) DCs for inducing the naïve T cell response.[62] We showed that a combination of lenalidomide and DC vaccination might synergistically enhance anticancer immunity in the murine myeloma model by suppressing immunosuppressive cells and by stimulating effector cells, as well as by effectively polarizing the Th1-specific immune response (unpublished data). These results suggested that modulation of the tumor microenvironment remains an important option for inducing the best anti-tumor immune response in MM.

FUTURE PERSPECTIVES ON DC-BASED CANCER IMMUNOTHERAPY

Although several disadvantages related to DC vaccines have been reported, DC-based vaccines are still a promising weapon in the treatment of cancers. Several aspects can be developed, such as the combination of multiple treatments and modification of tumor antigen and DCs themselves by use of molecular biology. Numerous recombinant proteins, such as Hsp,[63] carbonic anhydrase IX-Acinetobacter baumannii outer membrane protein A,[64] and HIV trans-activating[65] favor tumor antigen uptake, DC maturation, antigen cross-presentation, Th1 polarization, and CTL activity. Furthermore, several carrier systems such as liposome,[66] nanoparticle, immunostimulating complex, and virus-like particles[67] have been developed for antigen transport into the cytoplasm of DCs, inducing MHC class I presentation of tumor antigen on the DC surface. Genetic modification of DCs aims to express multiple epitopes in cytoplasm without any restriction on a patient's HLA type, up-regulation of co-stimulatory molecules, down-regulation of inhibitory molecules, modulation of Th1 cytokine secretion with low expression of regulatory cytokines, and promotion of recruitment of helper cells.[68] These approaches promise to make a potent DC-based vaccine that can be applied to eradicate tumors in the future.

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CONFLICT OF INTEREST STATEMENT

None declared.

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