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Cellular Immunotherapy Using Dendritic Cells Against Multiple Myeloma

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1. Introduction

Multiple myeloma (MM) is a clonal B cell malignant disease that is characterized by the proliferation of plasma cells in the bone marrow (BM) in association with monoclonal protein in the serum and/or urine, immune paresis, skeletal destruction, renal dysfunction, anemia, hypercalcemia and lytic bone diseases (Kyle & Rajkumar, 2004; Sirohi & Powles, 2004). Although the introduction of conventional chemotherapy, high-dose therapy with hematopoietic stem cell transplantation (HSCT), and the development of novel molecular target agents has resulted in a marked improvement in overall survival, the disease still remains incurable (Attal & Harousseau, 2009; Lonial & Cavenagh, 2009). Alternative approaches are clearly needed to prolong the disease-free survival as well as the overall survival of patients with MM. To prolong the survival of patients with MM who are undergoing allogeneic HSCT, donor lymphocyte infusion can be used successfully as a salvage therapy, which is based on the graft-versus myeloma effect in some cases of MM that relapse after allogeneic HSCT (Harrison & Cook, 2005; Perez-Simon et al., 2003). This role of immune effector cells 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 (Harrison & Cook, 2005).

DCs are the most potent APCs for initiating cellular immune responses through the stimulation of naive T cells. Immature DCs are good at antigen uptake and processing, but for a stimulatory T cell response they must mature to become fully activated DCs, which express high levels of cell surface-related major histocompatibility complex (MHC)-antigen and costimulatory molecules. Because of their ability to stimulate T cells, DCs act as a link in antitumor immune responses between innate immunity and adaptive immunity (Banchereau & Steinman, 1998). These DCs play a central role in various immunotherapy protocols by generation of cytotoxic T lymphocytes (CTLs) (Reid, 2001). DC-based vaccines have become the most attractive tool for cancer immunotherapy and have been used in the treatment of more than 20 malignancies, most commonly melanoma, renal cell carcinoma, prostate cancer and colorectal carcinoma (Palucka et al., 2011; Ridgway, 2003). In MM, cellular immunotherapy using DCs is emerging as a useful immunotherapeutic modality to

treat MM (Ridgway, 2003). Since tumor antigen-loaded DCs are expected to be able to stimulate tumor-specific CTLs and to overcome T cell tolerance in tumor patients, the development of DC vaccines that can consistently eliminate minimal residual neoplastic disease remains an important goal in the field of tumor immunology (Banchereau & Palucka, 2005).

2. Stream of DC research in MM

MM is believed to induce immunoparesis that interferes with DC function, which diminishes the effective antitumor immune responses in these patients. Usually, *ex vivo* DCs are generated from circulating blood precursors (i.e. monocytes) or bone marrow progenitor cells and are educated with tumor antigens prior to vaccination to patients. *Ex vivo* generated DCs can be loaded with myeloma-associated antigens as vaccines for patients with MM. The use of immature DCs or mature DCs, the way to induce DC maturation, types of tumor antigens, the techniques to load tumor antigens to DCs, routes of administration, dosing schedules are being investigated (Figdor et al., 2004; Nestle et al., 2001).

2.1 Idiotype and idiotype-pulsed DCs in MM

B cell malignancies are distinct from other types of cancer in that a tumor-specific antigen can be defined, namely the variable (V)-regions of the monoclonal immunoglobulin (Ig) that each B cell tumor clone produces. These V-region antigenic determinants are called idiotopes, and the sums of the idiotopes represent the idiotype (Id) of the monoclonal Ig. Id has distinct advantages as a tumor-specific antigen, which can be readily isolated from the plasma of MM patients (Hart & Hill, 1999). The Id protein has been used for immunotherapy both *in vitro* and *in vivo* in MM, and has demonstrated a successful response in follicular lymphoma and a unique expression of Id on the malignant B cell clone (Bergenbrant et al., 1996; Kwak et al., 1995). The first reported in 1971 demonstrated that Id is immunogenic in mice of the same inbred strain in which the myeloma cell originally developed (Sirisinha & Eisen, 1971). In addition, Id vaccination could induce both antibody and Id-specific T cells including CD4⁺ T cell and CD8⁺ T cell response by the presentation of Id protein on MHC class I and II of professional APCs. Id-specific CD4⁺ T cells appeared to be more frequent than CD8⁺ cells to response against Id protein. Id-specific CTL lines could be generated that killed autologous primary myeloma cells *in vitro*, and killing activity was induced by only MHC class I - restricted (Li et al., 2000), while in the other report both class I - and class II - restriction was observed (Wen et al., 2001). In MM, a number of studies using id vaccination in alone or in combination with cytokines and/or conjugate has been investigated. The Id protein was used as an autologous myeloma protein either alone (Bergenbrant et al., 1996) or combination with cytokine IL-2 with or without granulocyte-macrophage colony-stimulating factor (GM-CSF) (Hansson et al., 2007; Osterborg et al., 1998; Rasmussen et al., 2003) or conjugation with keyhole limpet hemocyanin (KLH) to vaccinate myeloma patients (Coscia et al., 2004; Massaia et al., 1999). In general, Id-specific responses were observed with variable frequency, in which T cell and B cell responses were detected *in vitro* following Id vaccination, but clinical responses were unsatisfactory and the long time response was not observed.

Autologous DCs that were generated from MM patients have been shown to efficiently endocytose different classes of Id protein, and autologous Id-specific CTLs lines containing both CD4⁺ and CD8⁺ T cells that were generated by Id-pulsed DCs significantly recognized and killed the autologous primary myeloma cells *in vitro* (Butch et al., 2001; Wen et al., 2001). Until now, the various studies of DC-based Id vaccination in MM have been reported (Bendandi et al., 2006; Lim & Bailey-Wood, 1999; Liso et al., 2000; Reichardt et al., 2003; Rollig et al., 2011; Titzer et al., 2000; Yi et al., 2002, 2010). Although Id-specific CTLs and immune response could be induced in some patients, clinical responses have been observed rarely in few patients after vaccination (Titzer et al., 2000). The Id-pulsed DC in combination with KLH (Liso et al., 2000; Yi et al., 2010), cytokine IL-2 (Yi et al., 2002) were used for vaccination in MM patients to improve the effectiveness of Id-pulsed DC vaccine. However, even both cellular and antibody responses have been observed, the clinical response also was not improvement following vaccinations. The reasons for these results may be attributed mainly to the Id protein as a weak antigen, and the use of immature DCs in some studies (Lim & Bailey-Wood, 1999; Osterborg et al., 1998; Wen et al., 1998).

2.2 Myeloma-associated antigens-based DC immunotherapy

Tumor-associated antigens (TAAs) have been identified in many tumor types including solid tumors and hematological malignancies. The highly specific TAAs such as the antigen that are present in one or only a few individuals and not found in normal cells for a particular tumor, or are only present in a number of related tumors from different patients or overexpress in increasing amounts in malignant cells were the greatest potential for clinically useful assays. Successful immunotherapy requires these sources of TAAs, which provide immune responses against the tumor cells or the cancer tissues that express the TAA on the tumors. A variety of myeloma-associated antigens have been identified in MM patients, which possibility provides an immune response by DC-based vaccine. Many potential TAAs in MM have been investigated including polymorphic epithelial mucin (MUC1), human telomerase reverse transcriptase (hTERT), PRAME, HM1.24, SP17, Wilms' tumor I (WTI), Dickkopf-1 (DKK1), heat shock protein (HSP) gp96 or member of cancer germ-like family (MAGE, GAGE, BAGE, LAGE, NY-ESO-1) (Batchu et al., 2005; Brossart et al., 2001; Hundemer et al., 2006; Lim et al., 2001; Qian et al., 2007; Szmania et al., 2006). T cells from myeloma patients can recognize a variety of TAAs, which suggesting that the T-cell has the capacity to kill myeloma cells selectively if these clonal populations can be activated and expanded effectively by a potent TAA. Among the various TAAs, some have been tested as peptide vaccines and only a few of them has been tested *in vitro* to induce TAA-specific CTLs response via loading the potent TAA to DCs in MM. The first TAAs pulsed with DCs in MM was MUC1, which was expressed on all of MM cell lines and primary myeloma cells and in sera of MM patients. Vaccination with MUC1 antigen has not been studied in MM patients, but 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 (Brossart et al., 2001; Milazzo et al., 2003). NY-ESO-1 is the most immunogenic of the cancer testis antigens, which are expressed in a variety of tumors, while their presence in normal tissue is limited to the testis and placenta (Szmania et al., 2006). In MM, expression of NY-ESO-1 has been correlated with more advanced disease (van Rhee et al., 2005). Spontaneous humoral and CD8⁺ T cell-mediated responses to NY-ESO-1 have been identified in patients with advanced disease

(Szmania et al., 2006; van Rhee et al., 2005). Although the clinical trial using NY-ESO-1 with DCs has not been tested, the *in vitro* monocyte-derived DCs transduced with the PTD-NY-ESO-1 protein can induce CD8⁺ cellular antitumor immunity superior to that achieved with NY-ESO-1 protein alone (Batchu et al., 2005). Sperm protein 17 (Sp17), the other immunogenic TAA, has been used as a tumor antigen to load into DCs. Sp17-specific HLA class I-restricted CTLs were successfully generated by DCs that have been loaded with a recombinant Sp17 protein and the CTLs were able to kill autologous tumor cells that expressed Sp17 (Chiriva-Internati et al., 2002). The over-expression of hTERT on MM compared to normal cells indicated that this telomerase could be used as tumor antigen to induce antitumor immune responses (Vonderheide et al., 1999). hTERT was capable of triggering antitumor CTL responses and kill hTERT⁺ tumor cells (Vonderheide et al., 1999). Recently, the activated T lymphocytes that were stimulated by DCs loaded with hTERT- and MUC1-derived nonapeptides were successfully able to kill myeloma cell line (Ocadlikova et al., 2010). DKK1, a novel protein that is not expressed in most normal tissues but is expressed in almost myeloma cells, could be a potentially important antigenic target for antimyeloma immunotherapy (Qian et al., 2007). DKK1-specific CTLs that were generated by DCs pulsed with DKK1 peptides were specifically lysed autologous primary myeloma cells and DKK1-positive cell line (Qian et al., 2007). In general, TAAs could be a major interest in immunotherapy in MM. However, problems that should be solved before starting the clinical trials include defining whether a specific TAA is a suitable and safe for immunotherapy of patients with MM. One problem was that TAA susceptible of inducing autoimmunity provided that autoimmunity remains limited to some tissues or is controllable. The other problem was that some members such as Sp17 and MUC1 have been detected in normal tissues; therefore, it remains to be elucidated whether specific CTLs are able to recognize only myeloma cells. Furthermore, although the other TAA such as PRAME and Sp17 could be over-expressed on almost MM cell lines, only a small number of tumor samples from MM patients showed a similar level, limiting its usefulness as an isolated TAA in MM. To overcome the effect of TAAs-based immunotherapy, trials involving more than one TAA need to be designed. Taken together, the data support DC immunotherapy with TAAs as being a promising immunotherapy to support to clinical trials in MM.

2.3 Whole tumor antigen-based DC immunotherapy

An alternative to Id protein- or TAA-based immunotherapy in MM is to use other tumor antigens that derived from whole tumor preparation to improve the efficacy of the DC vaccination in patients with MM. Although a single TAA has the possibility to induce the antitumor immune responses against MM, tumors may escape immune recognition by down-regulating expression of a particular antigen. In contrast, DCs loaded with antigens derived from whole tumor cells can improve the antitumor response and that limits the risk for immunological escape. There have been increasing reports of these alternative approaches, such as DCs pulsed with myeloma lysates (Hayashi et al., 2003; Lee et al., 2007; Wen et al., 2002), DCs pulsed with myeloma apoptotic bodies (Nguyen-Pham et al., 2011; Yang et al., 2010; Yang et al., 2011), DCs transfected with myeloma-derived RNA (Milazzo et al., 2003), DCs pulsed with myeloma-derived HSP gp96 (Qian et al., 2005; Qian et al., 2009), or DC-myeloma cell hybrids (Gong et al., 2002; Hao et al., 2004; Vasir et al., 2005). These techniques have the advantage of allowing the presentation of multiple epitopes to MHC on DCs, therefore can induce polyclonal T cell response from many potentially unknown TAAs

and reduce the probability of immune escape by single TAA. The first study reported that bone marrow mononuclear cells from the patients with MM contained more than 90% CD138⁺CD38⁺ myeloma plasma cells and CTLs that were generated by DCs loaded with myeloma cell lysates demonstrated much stronger cytotoxicity against autologous plasma cells than did those by Id protein-pulsed DCs, which suggested the superiority of the myeloma cell itself as a source of a tumor antigen compared with the Id protein (Wen et al., 2002). In other myeloma model, DCs pulsed with purified and optimized myeloma cell lysate were shown to generate CTLs that killed autologous tumor cells but not against mismatch HLA cell lines or K562 cell lines *in vitro* (Lee et al., 2007). The apoptotic bodies derived from either myeloma cell lines or patient's myeloma cells also have been used as tumor antigen to loading with DCs. Interestingly, apoptotic bodies were shown to be more effective than cell lysate at inducing CTLs against autologous myeloma cells (Hayashi et al., 2003). Heat shock proteins (HSPs) are a class of functionally related proteins whose expression is increased when cells are exposed to elevated temperatures or other stress. Tumor-derived HSPs, such as HSP70 and gp96, are immunogenic and potent in stimulating the generation of tumor-specific CTLs. Myeloma-derived gp96 has been obtained and used to pulse DCs to generate the specific CTLs in MM. The specific CTLs was able to lyse myeloma tumor cells but not normal blood cells in a MHC class I-restricted manner and provide a rationale for gp96-based immunotherapy in MM (Qian et al., 2005; Qian et al., 2009). In other way, a promising vaccine strategy in which the autologous DCs were fused with patient-derived tumor cells has been developed. DC fused with tumor can stimulate both helper and cytotoxic T cell responses through the presentation of internalized and newly synthesized antigens (Vasir et al., 2005). In mouse MM models, vaccination with DCs fused with either myeloma cells or tumor cells that were genetically modified to express CD40L resulted in eradication of disease in tumor-bearing animal and protective against subsequent tumor challenge in animals (Gong et al., 2002; Hao et al., 2004). Recently, a phase 1 study in which patients with MM underwent serial vaccination with the DC fused with MM cell fusions in conjunction with GM-CSF (Rosenblatt et al., 2011) resulted in the expansion of circulating CD4⁺ and CD8⁺ lymphocytes reactive with autologous myeloma cells in 11 of 15 MM patients and a majority of patients with advanced disease demonstrated disease stabilization. In general, the production of DC vaccine by using whole tumor antigens has become promising in order to induce immunotherapy against MM.

3. Innovative researches in the field of DC vaccination

3.1 Immune disorder in MM

Usually, hematologic malignancies elicit measurable, albeit weak, immunogenic responses that are generally unable to mediate tumor destruction. They are able to escape immune surveillance by down-regulation of immune markers such as costimulatory molecules and MHC class I and II molecules as well as through the production of immunosuppressive cytokines by the tumor cells or by activation of suppressor cells such as regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs) (Kim et al., 2007). In particularly, MM induces immune paresis (Quach et al., 2010). Patients with MM have basically dysfunctional DCs that are functionally defective, evidenced by the decreased number of circulating precursors of DCs as well as impaired T cell stimulatory capacity (Brown et al., 2001; Ratta et al., 2002; Tucci et al., 2011). DCs in MM patients are a target of tumor-associated

suppressive factors, such as interleukin (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 (Brown et al., 2001; Ratta et al., 2002). In addition, the survival and proliferation of tumor cells is partially facilitated by the impaired endogenous immune surveillance against tumor antigens (Zou, 2005). Myeloma cells can produce immunoinhibitory cytokines, such as TGF- β , IL-10, IL-6 and VEGF, which play major roles in the pathogenesis of MM (Brown et al., 2001; Ratta et al., 2002). These tumor-derived factors can also modulate anti-tumor host immune responses, including the abrogation of DC function, by constitutive activation of the signal transducer and activator of transcription 3 (STAT3) (Yu et al., 2007). Impairment in both humoral and cellular immunity in MM is associated with impaired B cell differentiation and antibody responses (Brown et al., 2001), reduced T cell numbers specifically CD4⁺ T cells and abnormal Th1/Th2 CD4⁺ T cell ratio (Ogawara et al., 2005), impaired CTL responses (Maecker et al., 2003), and dysfunction of NK cells (Jarahian et al., 2007) and NKT cells (Dhodapkar et al., 2003). In addition, dysregulation of natural CD4⁺CD25⁺T regulatory (Treg) has been reported (Prabhala et al., 2006). Tregs are a group of immuno-suppressive T cells that have been implicated in the suppression of tumor immunity (Curiel, 2007). A higher number of Tregs were reported in myeloma capable of suppressive activity at T cell stimulation (Beyer et al., 2006). Recently, a human study reported that the proportion of CD4⁺FOXP3⁺ Treg cells was increased in MM patients at diagnosis and Treg cells from patients with MM were functionally intact as they were able to inhibit proliferation of both CD4 and CD8 T cells (Brimnes et al., 2010). More recently, the discovery of myeloid-derived suppressor cells (MDSCs) revealed these cells as potent suppressors of tumor immunity and, therefore, a significant impediment to cancer immunotherapy (Ostrand-Rosenberg & Sinha, 2009). MDSCs are a heterogeneous population of cells of myeloid origin, which are present and accumulate in most cancer patients and experimental animals with cancer, and which are considered as a major contributor to the profound immune dysfunction of most patients with sizable tumor burdens (Ostrand-Rosenberg & Sinha, 2009). MDSC levels in cancer patients are driven by tumor burden and by the diversity of factors produced by the tumor and by host cells in the tumor microenvironment (Gabrilovich & Nagaraj, 2009). MDSCs suppress antitumor immunity through a variety of diverse mechanisms (Gabrilovich & Nagaraj, 2009). MDSCs can suppress the activation of T cells, B cells, natural killer (NK) cells and NKT cells. In contrast, MDSCs can enhance the induction of Tregs. Antigen presentation is also limited by the expansion of MDSC at the expense of DCs. Recently, an increase in the proportion of CD14⁺HLA-DR^{-/low} MDSC in patients with MM at diagnosis was described, illustrating that this cell fraction is also distorted in patients with MM (Brimnes et al., 2010). Taken together, the immune paresis in patients with MM suggested that DC-based vaccine therapies in MM need to be boosted with other alternative approaches or potent DCs may be needed to increase the effectiveness of vaccination.

3.2 Key points to improve DC vaccination in MM

For improving clinical outcomes using DC-based immunotherapy, there have been increasing reports of alternative approaches, such as better cytokine combinations to

enhance DC function, effective tumor antigens to induce specific CTLs, or modifying signal transcriptions to overcome defective DC function. Our experience in the DC research field has revealed several key points to improve DC vaccination in cancer patients (Fig. 1).

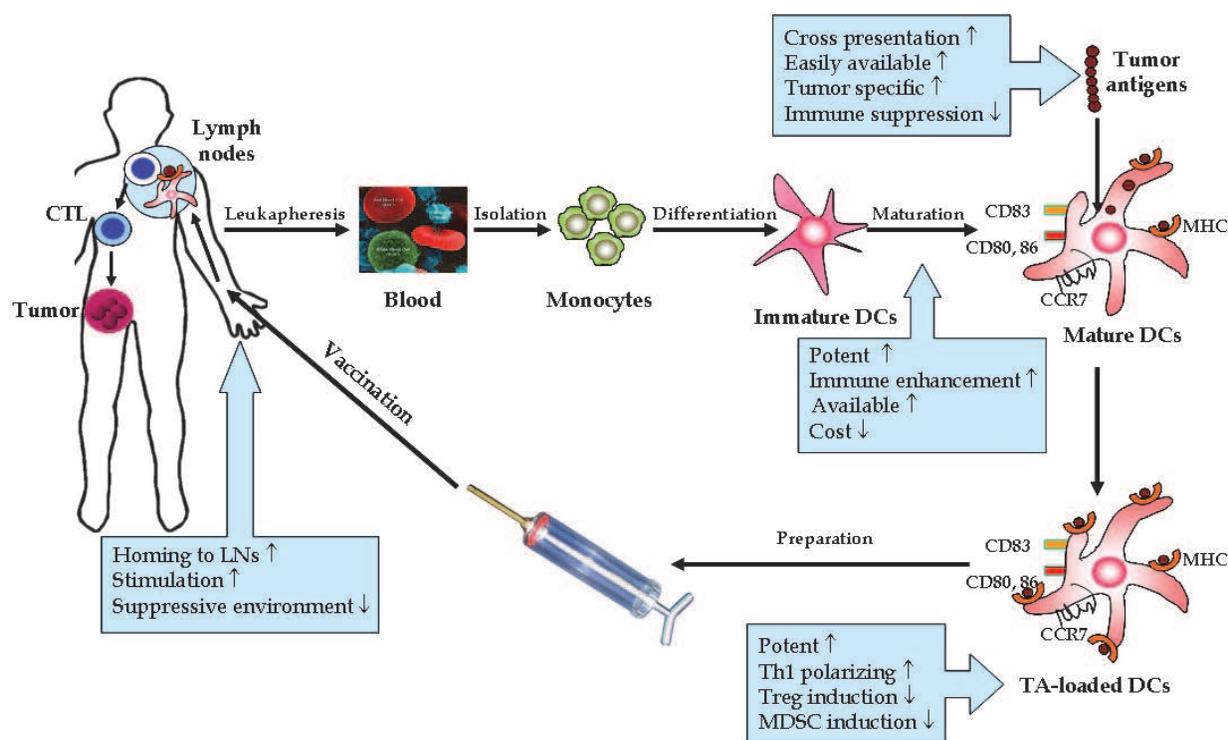


Fig. 1. **Key points to improve DC vaccination in cancer patients.** Abbreviations: CTL, cytotoxic T lymphocyte; DCs, dendritic cells; TA, tumor antigen; LNs, lymph nodes; Treg, regulatory T cell; MDSC, myeloid-derived suppressor cell.

As described above, the results of immunotherapy with Id-pulsed DCs have been unsatisfying. An alternative to Id protein is to use other tumor antigens that improve the efficacy of the DC vaccination in patients with MM. The selected antigen should possess the best characteristics to induce high cross presentation, be tumor specific, be easily available, but be unable to induce immune suppression. Whole tumor antigens is the best tumor antigen, which has been selected by many investigators including myeloma cell lysates (Kortylewski et al., 2005; Lee et al., 2007; Nefedova et al., 2005; Nefedova & Gabilovich, 2007; Wang et al., 2006; Wang et al., 2006), apoptotic bodies from myeloma cell line (Lee et al., 2007; Nguyen-Pham et al., 2011; Yang et al., 2010), and apoptotic allogeneic myeloma cells from other patients with matched subtype (Yang et al., 2011). In practical terms, there are a number of patients with MM, who have less than 50% of myeloma cells in the bone marrow at the time of diagnosis or during progression of the disease. When mononuclear cells from the bone marrow are used as a source of tumor antigens, there is the potential of contamination with normal cells, especially lymphocytes. Thus, it is necessary to use purified and optimized myeloma cells, if possible, as a source of tumor antigen for the generation of myeloma-specific CTLs stimulated by DCs (Lee et al., 2007). We have shown that the function of the DCs was affected by the concentration of myeloma cell lysates (i.e., higher concentrations of lysates suppress T cell stimulatory capacities more than lower concentration of lysates). Also, the optimization of the lysate concentration did not

demonstrate any inferiority in functions, such as T cell stimulatory capacities and cytotoxicities, of the DCs compared with other antigens, such as apoptotic bodies of myeloma cells or formalin-fixed myeloma cells. CTLs that were generated by purified and optimized myeloma cell lysates pulsed with DCs demonstrated much stronger cytotoxicity against autologous plasma cells. These findings indicate that it is important to optimize the concentration of myeloma cell lysates that were loaded onto DCs to potentiate their function.

The use of whole tumor cells, instead of single antigens, may help to enhance antitumor effects but target multiple tumor variants and counteract tumor immune evasion. However, it is impractical to obtain sufficient amounts of purified autologous myeloma cells for tumor antigens in the clinical setting of patients with MM. As an alternative source of tumor-relevant antigens, allogeneic tumor cells or established cancer cell lines have been used to overcome this limitation in various tumors (Koido et al., 2005; Lee et al., 2007; Palucka et al., 2006; Yang et al., 2010). Allogeneic myeloma cell lines used as universal tumor antigens could substitute for an original tumor cell collection and make the culture of tumor cells easier. In clinical practice, allogeneic myeloma cell lines might be an effective source of universal tumor antigen that could be used to load DCs for the generation of myeloma-specific CTLs in MM patients. Tumor antigens that derived from irradiated allogeneic myeloma cell line when loaded with DCs could generate myeloma-specific CTLs against autologous myeloma cells in patients with MM (Nguyen-Pham et al., 2011; Yang et al., 2010). These findings suggest that allogeneic myeloma cell lines are potent immunogens capable of inducing functional CTLs against patients' own tumor cells. The success of using an allogeneic myeloma cell line as tumor antigen led to the possibility that allogeneic myeloma cells could be also used as a viable source of tumor antigen in the context of appropriate major MHC alleles to autologous CTLs. We investigated the possibility of DC therapy using autologous DC loaded with apoptotic allogeneic myeloma cells from the matched monoclonal subtype of myeloma patients and showed that the CTL generated by these tumor antigens loaded-DCs could generate myeloma-specific CTLs against autologous myeloma cells in patients with MM (Yang et al., 2011). These findings suggested that the allogeneic matching monoclonal immunoglobulin subtype of myeloma is an effective tumor antigen capable of inducing functional CTLs against patients' own tumor cells.

The suppressive effects of tumor cells during DC generation have been explained previously by the ability of the tumor microenvironment to suppress DC differentiation (Savill et al., 2002; Yu et al., 2007). The suppression is due to the activation of STAT3 and the production of immunosuppressive factors, such as VEGF, IL-10, and IL-6. These factors can influence STAT3 and extracellular signal-regulated kinase (ERK) phosphorylation, resulting in hyperactivation of STAT3 and ERK, which may be responsible for defective DC differentiation (Kitamura et al., 2005; Yu et al., 2007). In addition to generation of potent and specific tumor antigen-loaded DCs for vaccination, alternative methods have attempted to restore defective DC function and to enhance DC function in MM. Enhanced immune-mediated antitumor effects of DCs have been reported following the inhibition of the janus-activated kinase 2 (JAK2)/STAT3 pathway (Nefedova et al., 2005), inhibition of p38 or activation of the MEK/ERK or mitogen-activated protein kinase (MAPK) pathways, and neutralization of IL-6 (Wang et al., 2006). Recently, we reported that the inhibitory factors and abnormal signaling pathways of DCs during maturation with tumor antigen might be

responsible for the defective activity of DCs in MM, and suggested that the way to overcome these abnormalities is by neutralizing the signaling that would lead to a suppressed immune response (Yang et al., 2009). More recently, we are developing of the strategies that recovering dysfunction of DCs caused from loading tumor antigen through the treatment of a combination of the selective JAK/STAT3 signaling pathway inhibitor (JSI-124) and the proteasome inhibitor (Bortezomib) onto myeloma cells (Lee et al., 2011). We reported that pretreatment of myeloma cells with combination of JSI-124 and bortezomib can recover DC dysfunction from loading the dying myeloma cells through the up-regulation of Hsp90 and the down-regulation of STAT3 phosphorylation and inhibitory cytokines production, and these DCs can generate to potent myeloma-specific CTLs.

For effective induction of tumor-specific immune responses in the field of DC vaccination, the DCs should have potency to stimulate T cells, to produce high levels of Th1 polarized cytokines (IL-12p70), to trigger Th1 polarizing capacity, and to migrate through lymphatic vessels to interact with T cells. Therefore, the strategy to generate the fully functional and potential DCs has been developed. The initial success of the therapeutic vaccines involving immature or partially-mature "first-generation" DCs has been reported (Hsu et al., 1996). However, such DCs express suboptimal levels of co-stimulatory molecules, and constitute a weaker immunogen than the subsequently-implemented mature DCs, constituting the "second generation" of clinically-applied DCs (sDCs). sDC vaccines induced by the IL-1 β /TNF- α /IL-6/prostaglandin E₂ (PGE₂) cytokine cocktail have been developed (Jonuleit et al., 1997). Such DCs are fully-mature DCs with high expression of co-stimulatory molecules, high expression of CCR7, and high migratory responsiveness to LN-associated chemokines; they have been widely tested in clinical trials. However, to date, the sDC vaccines have limitations that include the mediation of Th2 polarization, promotion of DC secretion of the immunosuppressive cytokine IL-10, inability to induce effectively the Th1-type response (because PGE₂ abolishes the secretion of IL-12p70), and high activity of such DCs in activating Treg cells (Banerjee et al., 2006; Kalinski et al., 1997, 2001; Yamazaki et al., 2006). Several investigators, including our group, have tried to develop the potent DCs for inducing effective tumor-specific immune responses. In an attempt to increase DC potency using cytokine combinations, α -type-1-polarized DCs (α DC1s) that are induced to mature using the α DC1-inducing cytokine cocktail IL-1 β , TNF- α , IFN- α , IFN- γ , and polyinosinic:polycytidylic acid [poly(I:C)] has been developed to generate strong functional CTLs in several diseases, on average 20-fold higher compared to sDCs (Lee et al., 2008; Mailliard et al., 2004). Recently, we successfully generated α DC1s from a patient with MM with high expression of costimulatory molecules, significant production of IL-12p70, and potent generation of myeloma specific CTLs (Yang et al., 2010, 2011). The potential of polarized α DC1s to produce IL-12 has important implications for the use of DCs as cancer vaccines.

The other strategy to induce potent DCs from patients with MM was the use of a "helper" cell to promote type 1 polarization of DCs. DCs and NK cells reciprocally activate each other during the immune response. Recent data from our and other groups demonstrate that such NK-DC interaction promotes the subsequent induction of tumor-specific responses of CD4⁺ and CD8⁺ T cells, allowing NK cells to act as "helper" cells in the development of the type 1 DCs in responses against cancer (Mailliard et al., 2003; Nguyen-Pham et al., 2010, 2011). Resting NK cells that are activated in the presence of TLR agonist, IL-2, and IFN- α can

induce DCs from patients with MM maturation and enhance IL-12p70 production *in vitro*. These potent DCs can be developed to generate strong functional CTLs against myeloma cells compared to sDCs (Nguyen-Pham et al., 2011).

Therapeutic DC vaccines against cancer not only need to be highly effective in inducing the expansion of tumor-specific T cells, but they also need to avoid interaction and induction of Tregs. Recently, the type 1-polarized DCs were demonstrated to suppress the secretion of CCL22 (Treg and Th2 type attracting chemokines), enhance the secretion of CCL5 and CXCL10 (Th1 and effector T cell-attracting chemokines), and suppress the induction of Tregs compared to sDCs or PGE₂-matured DCs (Muthuswamy et al., 2008). DCs generated *in vitro* for vaccination protocols that can target a local lymph node are highly sought, but difficult to achieve in practice. Type 1-polarized DCs, with higher levels of IL-12p70 and potent CTL generation targeting, are, however, limited by their migratory capacity to primary lymph organs due to the relatively lower expression of CCR7 compared to sDCs. We recently reported on the nature of the enhancement of the migratory phenotype of DCs. The first important mediator in the mobilization of DCs to lymph nodes is CCR7. However, upregulation of CCR7 alone by DCs is insufficient to drive DC migration toward CCL19 and CCL21. Up-regulation of CD38 and down-regulation of CD74 regulate DC migration *in vitro* and *in vivo* (Faure-Andre et al., 2008; Frasca et al., 2006). By regulating CD38, CD74, and CCR7 expression on DCs, type I and II IFNs have synergistic effects in the presence of TLR agonists on the regulation of DC migration and may provide a novel approach to improving vaccination efficacy (Nguyen-Pham et al., 2011).

Finally, to enhance the antitumor effectiveness of DC-based vaccines in preclinical *in vivo* mouse models, we have developed several models of combination therapy of DCs with an immunomodulatory drugs, such as cyclophosphamide or lenalidomide. Cyclophosphamide is frequently used to enhance or augment the antitumor effects in cancer immunotherapy (Ghiringhelli et al., 2004; Mihalyo et al., 2004). The possible effect of cyclophosphamide to enhance the antitumor efficacy of DC vaccine may be due to the increasing proportion of IFN- γ secreting lymphocytes in combination with the suppressing proportion of CD4⁺CD25⁺FoxP3⁺ Treg cells in tumor-bearing mice (Liu et al., 2007). The result of a clinical trial using allogeneic DC vaccine combined with low-dose cyclophosphamide has revealed that the combination therapy could induce stronger antitumor response compared with DC vaccine alone (Holzl et al., 2005). Recently, we developed a combination therapy in mouse cancer model which showed that a single administration of low-dose cyclophosphamide before the first DC vaccination augmented the antitumor effects of DC vaccine to eradicate tumor completely and consequently prolonged the survival of vaccinated mice (Pham et al., 2010). We are now developing a clinical trial in MM patients using this combination therapy.

4. DC-based vaccine in published clinical trials

Clinical trials of DC-based vaccine for MM have been restricted until now. The trial protocol and responses are summarized in Table 1. Almost of the clinical trials were related with using Id-pulsed DC alone or in combination with adjuvant such as cytokines or KLH. In the decade after the first DC-based Id vaccination was started at Stanford University, the results of clinical trials were limited. In general, the majority of clinical trials conducted using Id-pulsed DCs showed immune responses. However, the clinical responses were

unsatisfactory, mainly due to the poor immunogenicity of the Id protein. More recent results demonstrated improved clinical response by DC-based Id vaccination (Rollig et al., 2010; Yi et al., 2010). Therefore, DC-based Id vaccination is a possible way to induce the specific T cell responses in myeloma patients. Further trials with increasing numbers of patients are needed to increase the rate of responses.

Most recently, a phase I study was undertaken, in which patients with MM were vaccinated with an autologous DC/tumor cell fusion in combination with GM-CSF administration on the day of DC vaccination (Rosenblatt et al., 2011). Vaccine generation was successful in 17 of the 18 patients. The expansion of circulating CD4⁺ and CD8⁺ T cells reactive with autologous myeloma cells in 11 of 15 evaluable patients were detected. A majority of patients (11 of 16) with advanced disease demonstrated disease stabilization, with three patients showing ongoing stable disease at 12, 25, and 41 months. Interestingly, antibody response against some TAAs, such as regulators of G-protein signaling 19 (RGS19), HSP90, BRCA1-associated protein (BRAP) was also detected. So, vaccination with DC/MM fusions was feasible and may provide a new source of DC-based vaccines for the development of immunotherapy against MM.

A commercial product is currently being tested in a phase III trial (Mylovenge™, Dendreon Corp, Seattle, WA, USA). Mylovenge (APC8020) is conducted by pulsing autologous DCs with the patient's Id. A recent report of this commercial product showed that the long-term survival of those receiving the vaccine compared to all other patients with MM who underwent autologous HSCT (Lacy et al., 2009). This approach needs further testing in a phase III trial to confirm the clinical response and define the role of this DC vaccine in MM. We are also conducting a phase I/II clinical trial using type 1-polarized DCs loading with tumor antigens derived either from allogeneic myeloma cell line or patient's autologous-/allogeneic- myeloma cells in combination with chemotherapy in patients with MM after autologous HSCT.

5. Future perspectives

Despite their relative limitations, the data from recent clinical studies have suggested that DC-based vaccine may be a potential therapy in inducing the rate of tumor responses and prolonging the survival of patients with MM. In an attempt to increase DC-based potency and improve immune responses following vaccination, further investigations of additional tools to identify the alternative tumor antigens uniquely or specifically expressed on myeloma cells are needed, to recover or restore the dysfunction of DCs in MM patients, to induce T cells with the desirable effector functions rather than regulatory functions, to migrate into lymph nodes to stimulate T cells, and to clarify the ability of tumor specific CTLs to recognize and kill tumor cells. In our expectation, type 1-polarized DCs can be developed to generate strong functional CTLs. The allogeneic myeloma cell lines or allogeneic myeloma cells might be an effective source of universal tumor antigen that could be used to load to the DC1s for the successful generation of myeloma-specific CTLs. Eventually, the combination therapy, in which a DC vaccine is combined with either alternative therapy including chemotherapy, radiation therapy, molecular target therapy or other immunotherapy (adoptive therapy, NK cells therapy) or with adjuvant, will provide vigorous and maintained immune responses with the benefit clinical efficacy.

Author	DC type	TA	Adjuvant	Immune responses	Clinical responses
Liso et al.	imDC	Id	± KLH	4/24 Id-specific	17/26 SD
Lim et al.	imMo-DC	Id	KLH	5/6 Id-specific; 2/6 Id-specific IFN- γ ; 3/6 increase in Id-specific CTL frequency	6/6 PD
Reichardt et al.	imDC	Id	none	2/12 Id-specific proliferation; 1/3 Id-specific CTL	2 relapse; 8/10 PD; 2/10 SD
Titzer et al.	CD34-DC	Id	none	4/10 Id-specific T cell proliferation; 1/10 decreased BM plasmacytosis	1/10 SD; 9/10 PD
Cull et al.	imMo-DC	Id	none	2/2 Id-specific T cell proliferation; no Id-specific CTL response	2/2 PD
Yi et al.	mMo-DC	Id	Il-2	2/5 Id-specific T cell proliferation; 5/5 Id-specific B cell proliferation; 4/5 Id-specific IFN- γ	1/3 PR; 3/5 SD; 1/5 PD
Bendandi et al.	mMo-DC	Id	none	4/4 anti-KLH response; 2/4 Th1 cytokines response	1/4 SD; 3/4 PD
Lacy et al.	APC8020 (Mylovenge)	Id	none	None reported	6/26 CR; 2/26 PR; 19/27 SD Overall survival: 5.3 years of follow-up for alive patients
Lacy et al.	CD40 L-DCs	Id	KLH	9/9 Id-specific IFN- γ ; 5/9 Id-specific CTL response; 8/9 anti-KLH response	6/9 SD; 3/9 slowly PD; 4/6 continue SD after 5 years
Rosenblatt et al.	DC/tumor fusion		GM-CSF	11/15 CD4 and CD8 response with autologous myeloma cells; 5/5 tested anti-MUC1 response	11/16 SD (3/11 > 1 years SD; 8/11 2.5-5 months SD)
Rollig et al.	mMo-DC	Id	KLH	5/9 Id-specific T cell proliferation; 8/9 Id-specific cytokines response;	3/9 M protein decrease; 5/9 M protein stable

Table 1. **Summary of Clinical trials of DC-based vaccine for MM.** Abbreviations: DC, dendritic cell; TA, tumor antigen; imDC, immature DC; Mo-DC, monocyte-derived DC; Id, idiotype; mMo-DC, mature Mo-DC; KLH, keyhole limpet hemocyanin; CTL, cytotoxic T lymphocyte; PD, progressive disease; PR, partial response; SD, stable disease; CR, complete response

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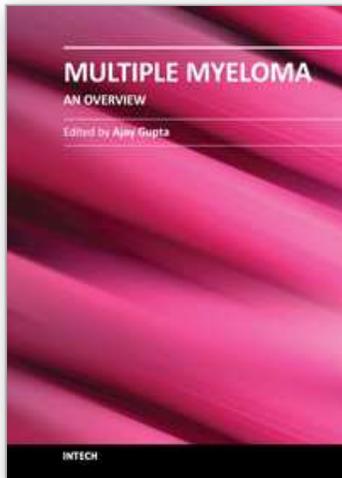
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Multiple Myeloma - An Overview

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Multiple myeloma is a malignant disorder characterized by the proliferation of plasma cells. Much insight has been gained into the molecular pathways that lead to myeloma and indeed much more remains to be done. The understanding of these pathways is closely linked to their therapeutic implications and is stressed upon in the initial chapters. Recently, the introduction of newer agents such as bortezomib, lenalidomide, thalidomide, liposomal doxorubicin, etc. has led to a flurry of trials aimed at testing various combinations in order to improve survival. Higher response rates observed with these agents have led to their integration into induction therapies. The role of various new therapies vis a vis transplantation has also been examined. Recent advances in the management of plasmacytomas, renal dysfunction, dentistry as well as mobilization of stem cells in the context of myeloma have also found exclusive mention. Since brevity is the soul of wit our attempt has been to present before the reader a comprehensive yet brief text on this important subject.

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