Chapter 6
Dendritic Cells and Peptide-Based Vaccine In Multiple Myeloma

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6.1 Introduction

Since Steinman and Cohn [1]'s initial report on dendritic cells (DC) with a distinctive stellate morphology, DC have been extensively studied by many other investigators for their major role as antigen-presenting cells (APC) to stimulate T lymphocytes and induce the disease-specific cytotoxic T lymphocytes (CTL). As major regulators of the adaptive immune response, DC have been known as the most potent APC for initiating cellular immune responses through the stimulation of naive T cells and to mediate antitumor responses in both preclinical studies and clinical trials [2–4]. The unique ability of DC to induce and sustain primary immune responses makes them prime candidates in vaccination protocols as a cancer therapy [5–8]. Therefore, translating the accumulating knowledge on DC subsets and their unique functional specializations into designs for novel vaccines is emerging as a key topic in the field of immunotherapy. More than 200 clinical trials have been performed using DC as cellular adjuvants in cancer [9]. The first US Food and Drug Administration approval in history for a therapeutic cancer vaccine was sipuleucel-T (Provenge; Dendreon, Inc.) that is an autologous DC-based vaccine loaded with a prostatic acid phosphatase (PAP)-GM-CSF fusion protein for treatment of men with advanced castrate-resistant prostate cancer. These ongoing studies have been accompanied by the development of a wide range of therapies using DC in other types of

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cancer, and we will specifically focus on the development of current DC therapies to treat multiple myeloma.

Multiple myeloma (MM) is a B-cell malignancy characterized by the clonal proliferation of malignant plasma cells in the bone marrow and the development of osteolytic bone lesions. Despite recent advances in treatment using new drugs, the disease still remains incurable; thus, novel approaches are required to improve therapeutic outcome [10–12]. In the post-allograft relapse setting, in which myeloma patients are chemotherapy refractory, long-lasting disease remission has been achieved after donor lymphocyte infusion (DLI) [13, 14]. Based on the success of allogeneic transplantation as well as graft-versus-myeloma responses following DLI, other types of immunotherapeutic approaches are being evaluated to treat the disease. The current focus has been on augmenting and directing autologous anti-MM immune responses as allogeneic immune manipulations put patients at risk of developing graft-versus-host disease with associated significant morbidity and mortality [15, 16]. It has been reported that the efficient generation of mature DC from peripheral blood $CD14^+$ monocytes in the majority of myeloma patients by culturing them with GM-CSF and IL-4 followed by TNF-α and/or other DC maturation factors can be utilized for immunotherapeutic purposes [17–22]. A number of approaches have been investigated including use of patient-specific idiotype, MM cell lysates, or MM cell-dendritic cell fusions.

6.2 Idiotype-Based DC Vaccine

Among the antigens identified on myeloma cells as potential targets, idiotype protein (Id) which is the immunoglobulin produced by myeloma cells has been investigated extensively [23–28]. The idiotypic determinants of the immunoglobulin are generated by rearrangement between the variable (V), diversity (D), and joining (J) regions in the heavy chain and between the V and J regions in the light chain. During maturation, a B cell may accumulate further diversity by somatic hypermutation [29, 30]. Tumor-specific Id secreted by MM cells can be easily detected in the blood of patients at concentrations which correlate to disease status [31]. Thus, the Id protein provides a clear tumor-specific antigen for B-cell tumors including MM and serves as a target antigen in various immunotherapeutic strategies. Several investigators have demonstrated that MM patients' T cells stimulated in vitro with Id-pulsed DC can kill autologous tumor cells in a MHC-restricted fashion and induce Th1-specific cytokines in vitro, thus demonstrating that MM cells process and present idiotypic peptides in the context of their MHC molecules and thereby can serve as targets of Id-specific T-cell-mediated antitumor responses [32–35].

Past and current clinical immunotherpies for MM patients have mainly been performed using Id as the antigen to boost patients' immune responses (Table 6.1).

Table 6.1 Idiotype-based clinical trials in myeloma

Vaccine	Patients(#)	Cellular responses	Clinical responses	Comments	Reference
Id alone	5	Increased T cell res	Insufficient CR	Show feasibility	Bergenbrant et al.
Repeated vaccination	I–III stage	3/5 ELISPOT	IFNg/IL4—2–5-fold up Anti-Id abs up		
Id + GM-CSF 6 shots	5	ELISPOTs IFNg/IL2 3–5 times up No IL4, proliferation 1/5 CD4/CD8 responded Class I restricted(46–100%) Class II restricted(5–37%) NO DTH responses	No clear CR	Paraprotein levels unchanged	Osterborg et al.
Id + KLH + GM-CSF (IL-2)	12 HD chemo autoSCT	Very little T-cell responses (2/11) No anti-Id abs w/ remission 85% had DTH	Residual tumor burden was not reduced after 36 m	Paraprotein levels not changed	Massaia et al. Coscia et al.
Id + IL12 ± GM-CSF 7 shots	6 stage I	5/6 up w T-cell responses	4/6 down tumor	No change in paraprotein	Rasmussen et al.
Id + GM-CSF 4 shots hepB Vac	3 autoSCT	T-cell responses up (1/3)	No alteration in	No abs to HepB	Bertinetti et al.
Tumor cell+ GMCSF K562 8 shots after paraprotein autoSCT	16	T/B-cell responses up antitumor abs up	1/16 CR/PR	3/16 rise in 4/10 had DTH 3/16 increased in	Borrello et al.
Id + KLH + GMCSF 52 in 3coharts	 18 BMT1/2+3shots 21 BMT1/2+6shots 13 BMT1+3shots+ BMT2+3shots	Prolif/cytokines up 14/18 7/19 7/11	 6/18 improved 12/21 improved 8/13 improved	 6/13 had DTH 5/10 had DTH 1/5 had DTH	Munshi et al.

In a pilot study by Lim and coworkers [36], six patients with IgG MM were vaccinated with intravenous infusions of DC derived from peripheral blood mononuclear cells (PBMC) pulsed with autologous Id protein. Although both a B-cell and a T-cell immune response were found, tumor-specific responses were only minor. In order to boost the Id-specific response, Reichardt and colleagues [37] conjugated myeloma-specific Id with keyhole limpet hemocyanin (KLH) and used the fusion protein to pulse autologous DC in vitro. They reported on 12 patients who had undergone autologous peripheral stem cell transplantation followed by a series of monthly immunizations of two intravenous infusions of Id-pulsed autologous DC and by booster immunizations with subcutaneous Id-KLH. This strategy was well tolerated as patients had only minor side effects. Furthermore, 2 of 12 patients developed Id-specific cellular proliferation, while 1 of 3 patients developed an Id-specific CTL response. In other studies, DC pulsed with Id-KLH have elicited potentially useful immunologic responses such as Id-specific T-cell proliferation detected from 15% [38] to as many as 83% of the patients [39] in clinical trials. In the latter study, the response was associated with production of IFN-γ in 2 out of 6 patients and an increase in CTL precursor frequency in these patients. In a study from Cull et al. [40], two patients with advanced refractory MM were vaccinated with Id-pulsed DC combined with GM-CSF. An anti-Id T-cell proliferative response was detected in both patients, which was also associated with IFN-γ production by the T cells. Titzer et al. [41] treated 11 patients with advanced MM with Id-pulsed, $CD34^+$ stem cell-derived DC and GM-CSF. Three of ten vaccinated patients showed an increased anti-Id antibody titer, and four of the ten patients had Id-specific T-cell responses.

Overall, meaningful immunologic responses and antitumor effects have been reported in lymphoma patients using different formulations of Id vaccine [42, 43]. However, the Id vaccination in B-cell cancers other than lymphoma is less advanced, and the vigorous Id-specific immune responses reported in lymphoma have not been detected yet in MM although DC-based Id vaccination can elicit Id-specific T-cell responses in patients with MM. This may be explained by the following aspects: (1) Id protein can induce humoral immunity; however, in contrast to lymphoma, myeloma cells do not express the IgG Id on the cell surface, and hence, the contribution of anti-Id antibodies to any vaccine-induced clinical response in myeloma is unclear [44]. (2) Early stage I myeloma patients with competent immune systems upon receiving DC-based Id vaccination displayed specific T-cell responses, and 89% of these patients demonstrated specific T-cell-mediated cytokine release after Id stimulation [26, 27]. In contrast, immune system suppression such as a functional defect in peripheral blood DC was observed in advanced myeloma patients when treated with Id-DC therapy [45]. In advanced myeloma, T-cell responses may be shifted to a type 2 inflammatory cellular response, and the functional activity of these T cells is a matter of debate [46, 47]. (3) Route of administration should be considered to help overcome the limitation of Id-pulsed DC vaccination. Most Id-pulsed DC vaccination trials have been administered intravenously [36, 37, 40, 41, 48]. However, several investigators report that

intravenous injection of DC led to accumulation of the cells in the lung, liver, and spleen during the first 24–48 h [49, 50], whereas DC injected subcutaneously migrated to the T-cell regions of draining lymph nodes and induced a strong protective immune response or a Th1-specific response [51]. In addition, Curti et al. [52] reported in a phase I/II clinical trial comparing subcutaneous and intravenous delivery of DC pulsed with Id that a more robust T-cell response was observed after subcutaneous DC injections along with increased Id-specific T-cell proliferation up to 1 year after vaccination in the myeloma patients. (4) Quality of DC should be explored in the clinical setting. Although monocyte-derived immature DC are both efficient in uptaking and processing antigens, the administration of these immature DC showed a limitation in triggering T-cell responses due to a lower expression of costimulatory and MHC molecules on their cell surfaces. In addition, monocyte-derived immature DC are not stable and may differentiate back to macrophages when IL-4 and GM-CSF are withdrawn [53]. In a study of functional differences between mature and immature DC, Yi et al. [54] concluded that mature DC derived from peripheral blood monocytes would better serve as APC than immature DC. Their clinical study using subcutaneous DC vaccination of Id-pulsed mature DC in MM patients with stable partial remissions following high-dose chemotherapy showed promising results, whereby Id-specific T-cell responses were observed in 80% of these myeloma patients. In a recent study, Yi et al. [25, 28] showed that intranodal administration of Id-pulsed CD40 ligand-matured DC induced Id-specific T-cell and B-cell responses in patients. (5) Several studies suggest that Id vaccination may have a therapeutic effect in the setting of autologous or allogeneic transplantation. Lacy et al. [55] showed that idiotype-pulsed DC following autologous stem cell transplantation for MM might be associated with prolonged survival. They demonstrated that 96% of the patients in the vaccine trial had achieved an objective response following autologous transplantation and suggested that Id vaccines are attractive as a consolidation therapy after autologous transplantation for MM. Exploitation of the potential antitumor effect of stem cell grafts in the allogeneic setting relies on strategies for enhancing graft-versus-tumor effects without aggravating graft-versus-host disease. In a study by Kwak et al. [56], donor-Id-specific T-cell immunity was detected at the time of allografting of Id-immune marrow. In another study, Li et al. [32, 35] showed release of high levels of Th1-type cytokines in an MHC-restricted fashion in response to stimulation with recipients' myeloma cells in two donors immunized with Id proteins obtained from their transplant recipients. These results set the stage for an ongoing phase I/II clinical trial at the National Cancer Institute of donor immunization prior to allogeneic stem cell transplantation followed by a nonmyeloablative conditioning regimen for MM. In the same clinical setting, to avoid any potential complications associated with immunization of healthy donors with tumor-derived products, in vitro priming of donor T cells using Id-pulsed DC may provide an alternative to in vivo donor immunization and allow the transfer of highly enriched populations of Id-specific T cells from donor to recipient [57] (Table 6.2).

Table 6.2 Clinical trials using DCs pulsed with myeloma patient idiotype

Vaccine	Patients	Clinical outcome	Reference
Id+KLH+ DCs 7 shots 2-iv Id+DC 5-Id+KLH	12 autoSCT hdose Chemo	Stable	Reichardt et al.
Id+DCs 3 shots	6	Progressed	Lim et al.
Id+ 4 shots GMCSF+ DCs	2 Adv refrat	1 progressed 1 stable	Cull et al.
2-Id+Dcs 7 shots 5-Id+KLH	26 hdose chemo autoSCT	17 live/stable	Liso et al.
1-Id+DC 4 shots 3-Id+GMCSF	11 III stage	Progressed	Titzer et al.
Id+DCs 3 shots IL2/5d	5 hdose chemo stable PR	4 stable 1 relapsed	Yi et al.
2-Id+DCs 7 shots 5-Id+KLH+ GMCSF	12 hdose chemo autoSCT at remission	10 progressed 2-PR	Reichardt et al.
alloDCs+Id Id+KLH+ GMCSF 4-7shots	4 RIC alloSCT	3 progressed	Bendandi et al. .
Id+DCs+ KLH 4 shots	9	All idiotype abs 5/9 CTL responses 3 progressed 4 stable	Yi et al.
Id+DCs 5 shots	9 stage I	5/9 anti-Idiotype abs 8/9 cytokine responses 3/9 dropped slightly	Rolliq et al.

6.3 DNA-Based DC Vaccine

Although proven effective in experimental models and in clinical trials, the traditional Id vaccine approach based on the culture of heterohybridomas is complicated in view of its clinical application by the need for large amounts of custom-made and individually tailored proteins that must be prepared and certified for each case within an appropriate time scale. The DNA vaccination technique provides ease of

vaccine generation and the specific protein production by host cells following immunization. The first requirement to make Id DNA vaccines is the identification of Id-encoding variable region genes (V_H and V_L) from tumor biopsies or blood. To construct Id DNA vaccines, the Id-encoding regions are isolated from malignant B cells using PCR-based techniques and formatted into a refined tumor-specific single-chain immunoglobulin (sFv) that retains the conformation of the native immunoglobulin. The weakly immunogenic, self-sFv is genetically fused to carriers, thus avoiding the need for purified Id protein, carriers, and adjuvants [58, 59]. For Id DNA vaccines, scFv alone was unable to reproducibly induce anti-Id antibody responses, even in the presence of the "immune stimulatory sequence" in the plasmid DNA backbone [60]. To improve the potency of Id DNA vaccines, investigators have constructed DNA fusion vaccines with scFv genetically linked to FrC, which is the nontoxic C fragment of tetanus toxin as an adjuvant to deliver a "danger signal" to the immune system [61, 62]. All of the fusion constructs were able to induce an antibody response against FrC in mice, and more importantly the linkage to FrC dramatically improved antibody responses against the patients' tumor IgM [63]. King and colleagues [64] further investigated a fusion DNA vaccine for induction of anti-Id responses and protection against challenge in syngenic mouse models, a surface Ig-positive lymphoma (A31) and a surface Ig-negative myeloma (5 T33). Their study showed that fusion of FrC enhanced anti-Id antibody responses, and the immunized mice were protected against tumor challenge in both cases. Lauritzsen and colleagues [65] have demonstrated that $CD4^+$ T cells are capable of protecting mice against challenge with a surface Ig-negative myeloma using anti-Id $CD4^+$ transgenic mice. The ability of scFv–FrC DNA fusion vaccines to induce an FrC-specific Th response suggests that the antitumor immunity observed by the fusion of FrC in the 5 T33 myeloma model may operate through the Th cooperation pathway [64].

A variety of different approaches have been explored using DNA fusion vaccines incorporating various immune-enhancing molecules or tumor-associated antigens (TAA) that can be used to promote immunity against attached tumor antigens. Different designs of these molecules can be used to circumvent tolerance and activate specific pathways of attack. Several investigators have developed a DNA vaccine approach using mediators of innate immunity such as proinflammatory chemokines or cytokines [66–68] and defensins [69] as genetic carriers, which deliver Id or a potential TAA to DC in vivo [70]. In two different mouse B-cell tumor models, this strategy converted Id into a potent immunogen with generation of both humoral and cellular antitumor immunity [69, 71]. Testing in pilot clinical trials showed insignificant toxicity, opening the way for the assessment of efficacy. Trudel et al. [72] in a phase I study evaluated the feasibility and safety of vaccinating MM patients after high-dose chemotherapy with adenovector-engineered, IL-2-expressing autologous plasma cells. These vaccines were well tolerated and induced a local inflammatory response consisting predominantly of $CD8^+$ T cells. However, no specific antitumor immunity or clinical responses were noted, and this indicates that further studies are needed to examine this clinical approach for treatment of patients.

6.4 Cell-Based DC Vaccine

A major drawback of an antigen-specific vaccine approach is that immune responses will be restricted to the single TAA with the subsequent risk of relapsing when tumors no longer express the antigens against which they were vaccinated, a phenomenon known as "antigen escape variants." An alternative to overcome this potential limitation is represented by whole tumor cell immunization (polyvalent vaccination), which may present to the host immune system a whole array of both known and as yet unidentified tumor antigens. This approach relies on the ability of the individuals' immune system to induce stronger immunity against tumor-selective antigens than against normal tissue antigens present on the tumor cells' surface.

Critical to this type of vaccine development is the ability to modify the tumor cell with genes encoding immunologically relevant molecules that produce a sustained, local release of its product, leading to a local inflammation at the vaccine site without systemic toxicity. Because of advances over the past decade in gene-transfer techniques, various tumor cells have been genetically modified to either secrete cytokines (e.g., IL-2, GM-CSF) or to express components of the cell membrane such as adhesion molecules or costimulatory molecules [73–77] that can enhance T-cell responsiveness. The means of active specific immunization using autologous tumor cells has been tested in trials for MM following their uptake and processing by DC in vivo. Trudel et al. [72] evaluated eight MM patients after vaccination with IL-2 expressing adenovirus engineered autologous plasma cells. Two months after high-dose therapy, six patients received from one to five injections of $3.5–9.0 \times 10^7$ of the engineered plasma cells. A phase I assessment found that the vaccine was effective in seven of eight patients with MM. Injection with tumor cells induced a local inflammatory response, and the clinical response, manifested as a decrease in serum paraprotein, was not observed in the one patient who had measurable disease at the time of vaccination. However, the limitation of this type of vaccine is that development of using cytokine-producing autologous tumor cells is hindered by the time needed for labor-intensive preparation of the vaccine and by the variability in the cytokine production of each patient's vaccine formulation. To overcome such drawbacks, investigators have developed an allogeneic bystander cell line (called K562) that secretes large and stable amounts of GM-CSF [78]. This cell line can be grown easily in suspension and has no detectable expression of HLA class I or class II molecules, and thus minimizes the likelihood of antibystander allogeneic responses with multiple vaccinations. This strategy of a universal bystander vaccine obviates the need for gene modification for each individual tumor source and ensures uniform cytokine production, thereby eliminating intrapatient and interpatient variability. In addition, GM-CSF produced at the vaccine site promotes the recruitment and activation of the host's APC, which efficiently uptake, process, and present tumor antigens to antigen-specific T cell, leading to strong antitumor responses.

In another effort to stimulate a broader antitumor immunologic response, investigators have explored the use of tumor lysate as a source of multiple antigens for vaccination. Wen et al. [79] demonstrated that patient-derived DC loaded with autologous tumor lysate induced antitumor immunity after repetitive stimulation in vitro. The T cells recognized and lysed autologous myeloma protein-pulsed DC and killed autologous primary myeloma cells. Another study also demonstrated the potent cytotoxic activities of CTL lines generated by DC pulsed with myeloma lysate against autologous target cells and showed the importance in the optimization of concentration of myeloma lysates utilized in pulsing of the DC [80]. Their results suggested that the DC pulsed with purified and optimized myeloma lysates could generate potent myeloma-specific CTL.

DC vaccines can also be made by fusing with myeloma cells. Several investigators have shown some efficacy using this vaccine approach in MM. Zhang et al. [81] showed that a DC-based tumor vaccine created by the formation of hybrid-engineered J558 tumor cells after fusion with DC induced an efficient tumor-specific CTL cytotoxicity against wild-type tumor cells in vitro and an efficient antitumor immunity in vivo. In other studies, investigators demonstrated that engineered J558 myeloma cells secreting IL-4, IL-12, or CD40 ligand, respectively, helped eradicate the established tumors [82–84]. They demonstrated that immunization of mice with the engineered fusion hybrid elicited stronger J558 tumor-specific CTL responses in vitro as well as more potent protective immunity against J558 tumor challenge in vivo than immunization with the conventional fusion hybrid DC/J558 created from the fusion of DC and unmanipulated J558 tumor cells alone. In addition, Grossman et al., [85] performed a DC fusion study using either primary myeloma cells from patients or a myeloma cell line (U266) and demonstrated that fusions with mature DC, as compared to immature DC, induced higher levels of T-cell proliferation and activation, as assessed by IFN-γ production and higher CTL activity against the myeloma cells. Tumor cell fusion has been known to induce maturation and the development of an activated DC phenotype necessary for their effectiveness as cancer vaccines [86]. Based on these results, a clinical trial was designed to evaluate the efficacy of vaccination of myeloma patients using fusion cells with myeloma cells and autologous mature DC. Rosenblatt et al. [87] have completed a phase 1 study in which patients with MM underwent serial vaccination with the DC/MM fusion product in conjunction with GM-CSF. Their study of vaccination was well tolerated, without evidence of toxicity and resulted in the expansion of circulating $CD4^+$ and $CD8^+$ T lymphocytes reactive with autologous myeloma cells in 11 of 15 evaluable patients. The vaccination with DC/MM fusions resulted in antitumor immune responses and disease stabilization in a majority of patients. In a separate report, they demonstrated that increased PD-1 expression was observed on T cells of patients with active myeloma compared with a control population of normal volunteers. However, it was returned to levels seen in normal controls, and anti-PD1 antibody enhances activated T-cell responses after DC/tumor fusion stimulation; thus, they suggested the potential enhanced vaccine efficacy in combination with the anti-PD1 antibody [88].

6.5 Peptide-Based Vaccines in Multiple Myeloma

6.5.1 Introduction

Active-specific immunotherapy has the distinct advantage of inducing highly effective T lymphocytes with antitumor activities [89, 90]. Long-term stabilization of disease with good quality of life has been demonstrated as a characteristic of cancer immunotherapy. To avoid a patient-specific immunotherapy requires individualized patient-specific products, which are labor intensive and costly, peptide vaccines can be used as an attractive therapeutic option for a broader applicability, low toxicity, and easy production [91]. Although there is MHC restriction in this therapeutic approach, use of cocktails of immunogenic peptides to different HLA molecules would broaden the induction of CTL specific to tumor cells of multiple MHC classifications. Based on the recent progress on the discovery of tumor-associated antigens (TAA), epitopes have been identified from multiple potential antigens and evaluated for the development of vaccines by eliciting the antigen-specific $CD8^+$ T-cell responses against MM cells. Strategies for further improvement in the efficacy of therapy, including combined use of chemotherapy drugs and molecular target-based drugs, are being proposed. Peptide vaccination in an "adjuvant setting" should be considered a promising treatment to protect against progression or relapse of malignancies in cases with minimal residual disease. The following are the types of TAA utilized and progress made for the development of peptide-based vaccines in MM.

6.5.2 Receptor for Hyaluronic acid Mediated Motility

Receptor for hyaluronic acid mediated motility (RHAMM) is an immunogenic antigen that is strongly expressed in several hematological malignancies including MM and induces humoral and cellular immune responses [92–94]. Schmitt et al. [95] and Greiner et al. [96] have investigated both immunological and therapeutic clinical responses to a RHAMM-R3 peptide vaccine in patients with MM. In their phase I trial, the RHAMM-R3 peptide (ILSLELMKL) was administered four times (300 μg or 1 mg/vaccination) subcutaneously at a biweekly interval to $HLA\text{-}A2^+$ MM patients who were in partial remission or near complete remission after high-dose chemotherapy with melphalan and autologous stem cell transplantation and had detectable free light chains in serum and/or urine and expression of RHAMM-mRNA in bone marrow or peripheral blood. Immune monitoring during or after vaccination for positive immune responses was performed on patient cells using the following criteria: (1) ELISpot analyses as an increase (>50%) in IFN-γ^+ and granzyme$^+$ spots, (2) tetramer analyses as an increase (>50%) in HLA-A2/R3-tetramer$^+$/$CD8^+$ T lymphocytes and with an increase (> 25%) in RHAMM-R3-tetramer$^+$/$CD8^+$ T lymphocytes, and (3) $CD8^+$ T-cell responsiveness demonstrated by a response 2/3

or 1/2 of the monitoring assays (tetramer staining, IFN-γ, and granzyme B ELISpot). Those patients having a positive immunological response showed an increase of $CD8^+$ $tetramer^+/CD45RA^+/CCR7^-/CD27^-/CD28^-$ effector T cells and an increase of RHAMM-R3-specific $CD8^+$ T cells. In addition, high-dose RHAMM-R3 peptide vaccination induced positive clinical effects. Two of four patients with MM showed a reduction of free light-chain serum levels.

6.5.3 Wilms' Tumor Gene

Wilms' tumor gene (WT1), which possesses oncogenic functions, is expressed in various kinds of malignancies. A series of investigations indicated that WT1 is a highly immunogenic antigen in patients with MM [97–99]. CTL epitopes were identified from WT1 specific to HLA-A2 and HLA-A-24, and evaluated in clinical trials. Vaccination of cancer patients with the WT1 CTL peptides induced immunological responses, which were assessed by ex vivo immunomonitoring, such as the tetramer assay, and in vivo immunomonitoring, such as the peptide-specific delayed type hypersensitivity reaction. The induced immunological responses then led to clinical responses as reduction of M-protein [100, 101]. The vaccination with a single WT1 peptide elicited an immunological response strong enough to induce a clinical response, suggesting that the WT1 peptide vaccine has therapeutic potential. The number of reports of the successful treatment of cancer patients with WT1 vaccination is increasing.

6.5.4 Dickkopf-1 (DKK1)

The DKK1 protein, a secreted protein and Wnt signaling pathway inhibitor, is produced by myeloma cells and overexpressed in myeloma microenvironment of patients with extensive bone disease [102, 103]. In addition to its direct inhibitory effect of DKK1 on osteoblasts, DKK1 disrupts the Wnt3a-regulated osteoprotegerin and receptor activator of NF-kappaB ligand (RANKL) expression in osteoblasts, and thus, it indirectly enhances osteoclast function in MM [104–107]. It is highly expressed by the tumor cells of almost all myeloma patients, and therefore, it has been suggested as an ideal target for immunotherapy in MM. However, DKK1 mRNA is detected in some normal tissues such as testis, prostate, placenta, and uterus, in addition to myeloma cells; thus, DKK1 resembles cancer–testis antigens because the most commonly used cancer–testis antigens NY-ESO-1 and MAGE are also found in the uterus, placenta, ovary, and even brain, in addition to tumors and testis [108, 109]. Qian et al. [110] identified an HLA-A2-specific peptide derived from DKK1 that was capable of inducing DKK1-specific T-cell lines and clones from $HLA\text{-}A2^+$ normal donors and MM patients. These CTL showed

peptide-specific and MM-specific responses in vitro and showed the therapeutic efficacy in vivo against established tumor cells in a HLA-A2 transgenic mouse model. These data show that DKK1 is a novel target for the management of myeloma patients with lytic bone disease.

6.5.5 Telomerase

Telomerase plays a critical role in cellular immortality and tumorigenesis. Its activity is normally not detectable in most somatic cells, while it is reactivated in the vast majority of cancer cells resulting in a tight correlation between telomerase activity and malignant potential of tumor cells [111–113]. Thus, inhibition of telomerase has been considered as a promising anticancer approach. Telomerase includes three major components: the telomerase reverse transcriptase (TERT) protein subunit that catalyzes the enzymatic reaction of DNA synthesis, the telomerase RNA (TR) component that serves as a template for TERT, and a protein termed dyskerin which binds to hTR. These three components are known to be essential for telomerase activity and telomere lengthening [114, 115]. Telomerase activity in a cell is associated with the expression of hTERT-related peptides on its surface and is present in more than 85% of human tumors [116, 117]. Recently, a multipeptide vaccine derived from the human telomerase reverse transcriptase (hTERT I540 (ILAKFLHWL), hTERT D988Y (YLQVNSLQTV), hTERT D988Y (YLQVNSLQTV)) and the antiapoptotic protein surviving (Sur1M2 peptide (LMLGEFLKL)) have been evaluated in a phase 1/2 two-arm trial [118, 119]. A total of 54 patients with myeloma received autografts followed by ex vivo anti-CD3/anti-CD28 costimulated autologous T cells at day 2 after transplantation. Study patients positive for HLA-A2 ($n=28$) also received pneumococcal conjugate vaccine immunizations before and after transplantation and the multipeptide vaccine. A subset of patients vaccinated (36%) developed immune responses to the tumor antigen vaccine by tetramer assays, but this cohort did not exhibit better median event-free survival (EFS). Adoptive transfer of tumor antigen vaccine-primed and costimulated T cells leads to augmented and accelerated cellular and humoral immune reconstitution, including antitumor immunity, after autologous stem cell transplantation for myeloma.

6.5.6 Cancer Testis Antigen

Cancer testis antigen (CTA) has been extensively studied in MM by many investigators. It exhibits physiological expression within germ cells and is frequently expressed in malignant tissue. Interestingly, immunological tolerance to CTA does not appear to be established, and the expression of CTA within malignant cells can therefore lead to induction of cellular and humoral immunity [120]. Antigen expression is detected most commonly in MM patients with advanced disease [121, 122],

but is also found in a significant proportion of patients with MGUS [123]. Recently, van Duin et al. [124] evaluated CTA expression in newly diagnosed MM patients ($n=320$) and in relapse cases ($n=264$) using Affymetrix GeneChips. They reported that relapse MM reveals extensive CTA expression and confirmed that the antigens are as useful prognostic markers in newly diagnosed MM patients and in relapse MM patients. The mechanisms that underlie this expression are unclear but are at least partially related to demethylation of gene promoter sequences [125]. DNA microarray analysis of gene expression of >95% pure myeloma cells from more than 300 patients showed that the genes of MAGE-3 and NY-ESO-1 were expressed in the tumor cells from patients with relapsed disease or abnormal cytogenetics [126]. The HLA-A1-restricted or HLA-A2-restricted MAGE-3- or NY-ESO-1-specific peptide have been identified and the tumor-specific CTL generated by the peptide were demonstrated against myeloma cells [127, 128]. In addition, MUC-1, HM1.24, and survivin are expressed on MM cells and have been shown to induce T-cell reactivity against the antigen in patients with MM [129–132]. Antigen-specific peptides have been identified from these potential target proteins [133–136] and have shown immunogenicity both in vitro and in vivo against myeloma cells. In a phase 1/2 two-arm trial, a combination of survivin and hTERT peptides was evaluated for their efficacy [118, 119]. The investigators showed that adoptive transfer of tumor antigen vaccine-primed and costimulated T cells leads to augmented and accelerated antitumor immunity after autologous stem cell transplantation for MM. In another study, MUC-1 and hTERT peptides were evaluated in vitro for their immunogenicity [137]. Following repeated stimulation of T lymphocytes with DC loaded with hTERT- and MUC1-derived nonapeptides, the resulting CTLs were identified by their high IFN-γ production. Next, these activated CTL were separated immunomagnetically, expanded in vitro, *and* tested for their cytolytic activity against a myeloma cell line. There were no statistically significant differences in the cytotoxic activities between the different antigen-specific CTL and their specific antigens expressed on MM cells. Christensen et al. [138] explored the possibility in vitro of using Melan-A peptide (aa26-35, EAAGIGILTV) with the hypothesis that Melan-A and Melan-A analog (ELAGIGILTV, aa26-35*A27L) peptide-specific T cells can be expanded reliably for immunotherapeutic application. They showed the ability of Melan-A analog (ELAGIGILTV, Melan-A (aa26-35*A27L))-specific T cells to recognize the HM1.24 (aa22-30: LLLGIGILV) peptide within the HM1.24 antigen presented by normal and malignant plasma cells. In addition, they found that Melan-A analog-specific T cells from HLA-$A2^+$ healthy donors and HLA-$A2^+$ MM patients secrete IFN-γ in response to HM1.24 (aa22-30) peptide-pulsed T2 cells. These peptide-specific CTL also lysed HLA-$A2^+$ $HM1.24^+$ U266 and XG-1 human MM-derived cell lines as well as the IM-9 B-lymphoblastoid cell line, and demonstrate that Melan-A analog-specific T cells cross-react with the HM1.24 peptide. Anderson et al. [139] discovered peptides derived from MAGE-C1 (CT-7), which is the most commonly expressed CTA found in MM. The CT-7-specific CTL recognizing two peptides targeted both MM cells as well as CT-7 gene-transduced tumor cells. They demonstrated that these epitopes are promising targets for developing an immunotherapy against myeloma or other CT-7^+ malignancies. In another study, Goodyear et al. [140] identified CTA-specific immune responses in patients with MM and

reported that recognition of HLA-B*0702-specific MAGE-A1 (289–298) peptide was the most dominant response seen with the their peptide panel. $CD8^+$ T-cell clones specific for the MAGE-A1 (289–298) peptide were isolated from three MM patients and demonstrated cytotoxic activity against MM cell lines. Interestingly, three clones from a HLA-B*0702-negative patient recognized the MAGE-A1 (289–298) peptide on a lymphoblastoid cell line expressing HLA-Cw7. The T-cell receptor gene usage was determined in five clones and showed conserved features in both α and the β chain genes indicating correlation between T-cell receptor usage and peptide specificity of CTA-specific T-cell clones. Clinical applicability of the peptides derived from the cancer–testis antigens is under evaluation.

6.5.7 XBP1

Besides CTA, other MM-associated antigens have been identified and evaluated as potential immunogenic epitopes for development of a vaccine therapy to treat MM. XBP1 is a basic leucine zipper-containing transcription factor, which is required for the terminal differentiation of B lymphocytes to plasma cells. To date, XBP1 is the only transcription factor found to be essential for plasma cell differentiation and immunoglobulin secretion. The expression of XBP1 is uniformly found in primary MM cells and cell lines, selectively induced by exposure to IL-6, and has been implicated in the proliferation of malignant plasma cells [141–144]. A splice variant of XBP1 has known to have a crucial role in normal plasma cell differentiation [145], and XBP1 splicing has been recognized to occur in terminal B-cell differentiation and correlates with plasma cell differentiation. Based on these observations, Bae et al. [146] proposed the XBP1 as a unique therapeutic target antigen and identified two heteroclitic peptides, YISPWILAV and YLFPQLISV, with improved HLA-A2-binding and stability from their respective native peptides, $XBP1_{184–192}$ (NISPWILAV) and XBP1 $SP_{367–375}$ (ELFPQLISV). CTL generated by stimulation of $CD3^+$ T cells with each HLA-A2-specific heteroclitic peptide showed an increased percentage of $CD8^+$ (cytotoxic) and $CD69^+/CD45RO^+$ (activated memory) T cells and a lower percentage of $CD4^+$ (helper) and $CD45RA^+/CCR7^+$ (naïve) T cells, which were distinct from the control unstimulated T cells. The CTLs showed functional activities and demonstrated MM-specific and HLA-A2-restricted proliferation, IFN-γ secretion, and/or cytotoxic activity in response to MM cell lines and primary MM cells. These data demonstrate the distinct immunogenic characteristics of unique heteroclitic XBP1 peptides, which induce MM-specific CTL.

6.5.8 CD138, CS1

Furthermore, Bae et al. ([147], 2012) introduced immunogenic peptides specific to CD138 and CS1 antigens, which offer additional targets to develop an

immunotherapy targeting MM. The CD138, also known as syndecan-1, is a transmembrane heparan sulfate-bearing proteoglycan expressed by most MM cells. It has cytoplasmic domain which is linked to cytoskeletal elements to potentiate anchorage of the cells and stabilize cell morphology, while their extracellular domain has up to three heparan sulfate chains that bind to numerous soluble and insoluble molecules. These associations include interactions with heparan-binding molecules on adjacent cells to mediate cell–cell adhesion, binding to molecules to mediate cell adhesion to the extracellular matrix, as well as binding to growth factors and cytokines; thus, CD138 is known to be critical for the growth of tumor cells [148, 149]. In patients with MM, shed syndecan-1 accumulates in the bone marrow, and soluble syndecan-1 is known to facilitate MM tumor progression, angiogenesis, and metastasis in vivo. Therefore, preventing or reducing high levels of syndecan-1 in the serum, an indicator of poor prognosis in MM [150–152], would have a direct clinical benefit by targeting CD138 on malignant plasma cells. A novel immunogenic HLA-A2-specific peptide, $CD138_{260\text{-}268}$ (GLVGLIFAV), identified by Bae et al. [147] induces antigen-specific CTL, and the CD138 peptide-specific CTL displayed a unique immunological phenotype, and HLA-A2-restricted responses and functional activities against both primary MM cells and MM cell lines expressing CD138 antigen. Additionally, CS1 (CD2 subset 1, CRACC, SLAMF7, CD319) has been utilized as a target antigen to potentially develop immunotherapy against MM. CS1 is a member of the signaling lymphocyte activating-molecule-related receptor family, which is highly expressed on MM cells and is absent in the vast majority of acute leukemia, B-cell lymphoma, and Hodgkin lymphomas [153]. In addition, CS1 antigen is not expressed by normal tissues or stem cells, but is expressed at low levels on NK cells and a subset of T lymphocytes compared with malignant plasma cells [153]. CS1 expression was observed on MM cells from all patients, including MM with high-risk and low-risk molecular profiles and those with and without cytogenetic abnormalities, suggesting that this antigen is not restricted to any particular MM subgroup [154]. Equally important for the development of immunotherapy, CS1 expression is maintained on patients' MM cells even after relapse of disease. Based on these findings, Bae et al. [155] identified a novel immunogenic HLA-A2-specific epitope, $CS1_{239–247}$ peptide (SLFVLGLFL), which is derived from the CS1 antigen and has the ability to evoke MM-specific CTL. The CS1 peptide-specific CTL demonstrated HLA-A2-restricted antitumor cytotoxicity and degranulation against HLA-$A2^+$ primary MM cells and MM cell lines. In addition, the specific CTL demonstrated cell proliferation and IFN-γ secretion in response to antigen restimulation, which is also HLA-A2 restricted and the antigen specific. They also observed distinct immunologic activities specific to MM cells within the CD8 effector memory ($CD45RO^-CCR7^-/CD3^+CD8^+$) T-cell subset, and proposed an immunotherapeutic approach using the $CS1_{239–247}$ peptide to effectively target MM cells and improve treatment outcome in patients with MM. These results highlight their potential application for immunotherapy to treat the patients with MM or its premalignant condition. Clinical applicability of the peptides derived from the antigens is under evaluation.

6.6 Future Directions

Active cancer immunotherapy has been proven to be an effective approach to induce T-cell immune responses and overcome a number of issues by passive cancer immunotherapy including the requirement for repeated dosing and its high cost, the development of resistance through loss of immunodominant epitopes and undesired immunogenicity of humanized or chimerized antibodies. Dendritic cell-based or peptide-based treatments have been proposed as promising candidates for development of active cancer immunotherapy by generation of TAA-specific CTL. Clinical trials with dendritic cell-based or peptide-based therapy in patients with MM show that the vaccinations were well tolerated and induced clinical benefit in the patients. However, the effectiveness of active cancer immunotherapy to induce the specific immune response and clinical benefit depends on several factors. Besides element of antigens, it is becoming critical to optimize various conditions of the immune system to generate a clinically effective antitumor response. Generally, vaccine alone is not sufficient to evoke a potent immune response. Future challenge for successful immunotherapy is to skew the immune response towards a Th1 and to increase the antigen-induced T cells that bear high-avidity T-cell receptor to the specific TAA by using optimal adjuvant. Adjuvant should be important to enhance the immune response through a wide range of mechanisms including a depot action causing slow release of antigen to local inflammation causing enhanced recruitment of antigen-presenting cells to the injection site and facilitation of cross priming and mimic a danger signal. Furthermore, administration of optimal cytokine would be supportive, not only for the activation and expansion of tumor-associated T cells, but also for potential induction of the migration of vaccine-induced circulating T cells to the tumor site. Importantly, it would be highly potential to reverse the tolerance to tumor by blocking the CTLA-4 or by depleting regulatory T cells. Additionally, type of antigen-presenting cell and its activation status in the subjects vaccinated should be considered for successful therapeutic outcomes by cancer vaccines. Clinical responses of active cancer immunotherapy have been shown as promising in patients with minimal residual disease; thus, the combination of tumor debulking treatment and vaccination has been considered as a potential strategy to lead a successful therapeutic outcome in patients. Lastly, the complexity of the immune network and of the interactions between the tumor and the immune system makes the task to optimize the regimen including vaccine dose and route and schedule of immunization.

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