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NOVEL IMMUNOTHERAPIES

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Abstract

Multiple myeloma is still a fatal disease. Despite advances in high-dose chemotherapy, stem cell transplantation, and the development of novel therapeutics, relapse of the underlying disease remains the primary cause of treatment failure. Strategies for post-transplantation immunomodulation are desirable for eradication of remaining tumor cells. To this end, immunotherapy aimed at inducing myeloma-specific immunity in patients has been explored. Idiotype protein, secreted by myeloma cells, has been the primary target for immunotherapy as it is the best defined tumor-specific antigen. This chapter focuses on novel immunotherapies that are being developed to treat patients with myeloma. I will discuss potential myeloma antigens, antigen-specific T cells and their function on myeloma tumor cells, and T-cell-based and antibody-based immunotherapies for myeloma. Furthermore, clinical studies of T-cell-based immunotherapy in the form of vaccination, allogeneic stem cell transplantation and donor lymphocyte infusions, with or without donor vaccination using patient-derived idiotype, and future application of donor-derived or patient-derived, antigen-specific T-cell infusion in this disease are also discussed. Based on the specificity of the immune effector molecules and cells, immunotherapies with specific T cells or therapeutic antibodies may represent novel strategies for the treatment of multiple myeloma in the near future.

Keywords

Multiple myeloma; immunotherapy; tumor antigens; antibodies; T cells

INTRODUCTION

Multiple myeloma (MM), characterized by the clonal expansion of malignant plasma cells, remains a fatal disease, and nearly 11,000 Americans die from the disease each year¹. MM constitutes 10% of hematologic malignancies in the United States and is more prevalent than lymphocytic leukemia, myelocytic leukemia, or Hodgkin disease. Despite advances in the treatment of MM by using conventional and novel therapeutics in combination with transplantation², long-term survival is rare and most patients will relapse and die of the disease^{2,3}. Thus, novel therapeutic approaches that have a mode of action different from and non-cross-resistant with cytotoxic chemotherapy are required to eradicate myeloma cells that have become multidrug resistant. Immunotherapy is an appealing option for this purpose^{4,5}.

Results from recent research have indicated that myeloma cells are susceptible to T-cell-mediated cytotoxicity. In the post-allograft relapse setting, in which myeloma patients are chemotherapy refractory, long-lasting disease remission has been achieved after infusion of donor lymphocytes, a phenomenon termed graft-versus-myeloma (GVM) effect^{6,7}. This

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GVM effect is closely associated with graft-versus-host disease (GVHD), and donor-derived alloreactive and tumor-specific T cells are believed to mediate these effects 6·7. These observations strongly suggest that chemotherapy and immunotherapy kill myeloma cells by different modes of action that are non-cross-resistant; therefore, they should work synergistically.

Clonogenic myeloma cells, either pre-plasmacytic or plasma cells, may include post-switch B cells. These cells are present in the bone marrow and peripheral blood of patients with MM. Myeloma B cells may express monoclonal immunoglobulin (Ig) on their cell surface, in addition to major histocompatibility complex (MHC) class-I and -II molecules and are sensitive to regulatory signals provided by cellular and humoral components of the idiotype-specific immune network 4·8·9. Plasma cells compose the major tumor burden and constitute at least 10%, but can be greater than 90%, of the total bone marrow cell count 1·10. Myeloma plasma cells secrete the monoclonal M-component and express cytoplasmic Ig 11–13. Moreover, myeloma plasma cells may express MHC class-I antigens 14–16; adhesion molecules, such as CD44, CD56, CD54, and VLA-4 17–19; signaling or costimulatory molecules CD40 and CD28 19·20; as well as the Fas antigen (CD95) 21,22. Some of the plasma cells also express HLA-DR, CD80, and CD86 15·23. Our study showed that myeloma plasma cells were able to activate alloreactive T cells and present the recalled antigens, purified protein derivative and tetanus toxoid, to autologous T cells 15. Therefore, myeloma plasma cells may also be subject to immune regulation, at least by the cellular components of the immune system.

TUMOR ANTIGENS FOR IMMUNE TARGETING IN MM

Idiotype proteins and idiotype-specific T cells

Idiotype proteins are tumor-specific antigens, and active immunization against idiotypic determinants on malignant B cells has produced resistance to tumor growth in transplantable murine B-cell lymphoma and plasmacytoma 24–28. The presence of idiotype-specific T cells in the peripheral blood of patients with MM or with the benign form of the disease, monoclonal gammopathy of undetermined significance (MGUS), has been studied by detecting idiotype-induced T-cell proliferation and cytokine secretion by using the enzyme-linked immunospot (ELISPOT) assay 29.

Idiotype-specific T cells at a low frequency were detected in 90% of patients with MM or MGUS 30–32. Consistent with these results, we and others have shown that T cells in myeloma patients responded to peptides corresponding to complementarity-determining region I–III of heavy and light chains of the autologous M-component 16,33–35. We found that idiotype-induced T-cell stimulation was mainly confined to the CD4⁺ subset in most of the patients examined and was MHC class II-restricted. Idiotype-specific CD8⁺ T cells were also demonstrated, but at a lower frequency. Idiotype-specific CD4⁺ and CD8⁺ T cells were mainly of the type-1 subsets, as judged by their secretion of interferon (IFN)- γ and interleukin (IL)-2 36·37. Moreover, the proportion of individuals who had an idiotype-specific response of the T helper-1 (Th1)-type (IFN- γ - and/or IL-2-secreting cells) 38·39 was significantly higher in patients with indolent disease (MGUS and MM stage I) compared with those with advanced MM (stage II/III). In contrast, cells secreting the Th2-subtype cytokine profile (IL-4 only) 38·39 were seen more frequently in patients with advanced MM (stage II/III) 31. A similar pattern of cytokine secretion was also reported by others 40. Collectively, these findings indicate that the existing idiotype-specific immune response is too weak to control the growth of myeloma cells *in vivo* and that a shift from an idiotype-specific type-1 response, i.e., Th1 and T cytotoxic-1 (Tc1) 41, in early MM to a type-2 response (Th2 and probably Tc2 41) in advanced disease may have occurred. These studies provide indirect evidence that idiotype-specific T cells may have a regulatory impact on human tumor B cells.

To examine whether idiotypic-specific T cells can recognize and kill myeloma cells, we generated idiotypic-specific cytotoxic T lymphocyte (CTL) lines from myeloma patients⁴². To enhance the immunogenicity of idiotypic proteins, we used dendritic cells (DCs) as antigen-presenting cells. After repeated rounds of *in vitro* T-cell stimulation with idiotypic-pulsed autologous DCs, idiotypic-specific T-cell lines, which consisted of both CD4⁺ and CD8⁺ T cells, were generated and propagated from the peripheral blood mononuclear cells (PBMCs) of myeloma patients. Idiotypic-specific proliferative responses were observed when these T cells were rechallenged with the autologous, but not allogeneic, idiotypic-pulsed DCs. By using a standard ⁵¹chromium-release assay, our results showed that idiotypic-specific CTLs not only recognized and lysed autologous idiotypic-pulsed DCs but also significantly killed autologous primary myeloma cells. The cytotoxicity was MHC class I-, and to a lesser extent, class II-restricted, suggesting that myeloma cells could process idiotypic protein and present idiotypic peptides in the context of their surface MHC molecules. Taken together, these findings provide direct evidence that myeloma plasma cells express idiotypic peptides-MHC molecules on their surface and are susceptible to idiotypic-specific T-cell-mediated lysis.

Myeloma plasma cells and myeloma-specific T cells

Myeloma tumor cells may contain a multitude of tumor antigens that can stimulate an increased repertoire of anti-tumor T cells and lead to an induction of stronger antimyeloma responses. To explore the possibility of using myeloma cells as the source of tumor antigens for immunotherapy, myeloma cell lysate-specific CTLs were generated from patients by culturing T cells with autologous DCs pulsed with freeze-thaw lysate from myeloma cells⁴³. After four to six cycles of antigen stimulation, specific CTL lines containing both CD4⁺ and CD8⁺ T cells were obtained from four patients. These cell lines proliferated in response to autologous primary myeloma cells and DCs pulsed with autologous, but not allogeneic, tumor lysate and secreted predominantly IFN- γ and tumor necrosis factor (TNF)- α , indicating that they are type-1 T cells (Th1 and Tc1). The CTLs had strong cytotoxic activity against autologous tumor lysate-pulsed DCs and primary myeloma cells.

Myeloma-specific CTLs can also be induced and propagated by using myeloma-DC fusion cells as antigen-presenting cells. The heterokaryons generated by cancer-DC fusion cells combine the machinery needed for immune stimulation with presentation of a large repertoire of antigens. In murine plasmacytoma models, vaccination with DCs fused with mouse 4TOO plasmacytoma cells 44 or J558 myeloma cells⁴⁵ was associated with induction of anti-tumor humoral and CTL responses. Immunization with the fusion cells protected mice against tumor challenge and extended the survival of tumor-established mice without eradication of the tumor cells. In a more recent study, human myeloma cells, either primary myeloma cells from patients or a myeloma cell line (U266), were fused to human DCs⁴⁶. Fusions with mature, as compared with immature, DCs induced higher levels of T-cell proliferation and activation, as assessed by intracellular IFN- γ expression and stronger cytotoxic T-cell activity against the tumor cells. Alternatively, myeloma-specific CTLs could be generated *in vitro* by stimulating T cells with tumor-derived RNA-transfected autologous DCs⁴⁷.

DKK1 as a universal myeloma antigen

Dickkopf-1 (DKK1) is a secreted protein that specifically inhibits the Wnt/ β -catenin signaling by interacting with the co-receptor Lrp-6^{48,49}. Previous studies have shown that the *DKK1* gene has restricted expression in placenta and mesenchymal stem cells (MSCs) and not in other normal tissues^{50,51}. Recent studies demonstrated that DKK1 in myeloma patients was associated with the presence of lytic bone lesions⁵². Immunohistochemical analysis of bone marrow biopsy specimens showed that only myeloma cells contain detectable DKK1. Recombinant human DKK1 or bone marrow serum containing an elevated level of DKK1 inhibited the differentiation of osteoblast precursor cells *in vitro*. Furthermore, anti-DKK1

antibody treatment was associated with reduced tumor growth in myeloma mouse models 53–55. These results indicate that DKK1 is an important player in myeloma bone disease.

The identification of novel tumor-associated antigens, particularly those shared among patients, is urgently needed to improve the efficacy of immunotherapy for MM. For this purpose, we examined whether DKK1 could be a good candidate. We identified and synthesized DKK1 peptides for HLA-A*0201 and confirmed their immunogenicity by in vivo immunization of HLA-A*0201 transgenic mice. We detected low frequencies of DKK1 peptide-specific CD8⁺ T cells in myeloma patients by using peptide-tetramers and generated peptide-specific T-cell lines and clones from HLA-A*0201⁺ blood donors and myeloma patients. These T cells efficiently lysed peptide-pulsed but not unpulsed T2 or autologous DCs, DKK1⁺/HLA-A*0201⁺ myeloma cell lines U266 and IM-9, and more importantly, HLA-A*0201⁺ primary myeloma cells from patients. No killing was observed on DKK1⁺/HLA-A*0201[−] myeloma cell lines and primary myeloma cells or HLA-A*0201⁺ normal lymphocytes, including B cells (Figure 1). These T cells were also therapeutic in vivo against established myeloma in SCID-hu mice after adoptive transfer. These results indicate that these T cells were potent CTLs and recognized DKK1 peptides naturally presented by myeloma cells in the context of HLA-A*0201 molecules. Hence, our study identified DKK1 as a potentially important antigen for immunotherapy in MM.

Other antigens

Recent studies have shown that the cancer-testis antigens MAGE-3 and NY-ESO-1 may be expressed by myeloma cells 56–58. DNA microarray analysis of gene expression of >95% pure myeloma cells from more than 300 patients showed that the genes of these antigens were expressed in the tumor cells, particularly from patients with relapsed disease or abnormal cytogenetics (in 7–20% of MGUS and newly diagnosed MM and in 40–50% of relapsed patients or in patients with cytogenetic abnormalities) 59–60. With the use of specific monoclonal antibodies (mAbs) against MAGE-3 or NY-ESO-1, it was evident that the proteins of these antigens were also expressed in the tumor cells of patients with positive gene expression. We then generated MAGE-3- and NY-ESO-1-specific CTLs from healthy individuals by using HLA-A1-restricted or HLA-A2-restricted MAGE-3-derived and NY-ESO-1-derived synthetic peptides as the antigens with which to pulse autologous DCs. MAGE-3-specific CTLs killed peptide-pulsed autologous target cells and MAGE-3- and HLA-A1-positive myeloma cells (line ARK-RS). No killing was observed with K562 cells, unpulsed target cells, or myeloma cell lines that were HLA-A1-positive but MAGE-3-negative 61. Similar results were obtained with NY-ESO-1-specific CTLs 62.

Furthermore, other antigens, such as MUC-1 63–65, sperm protein 17 (Sp17) 66,67, and HM1.24 68–70, may also be expressed on myeloma cells, and MHC-restricted antigen (MUC-1 71 and Sp17 72)-specific CTLs have been generated from myeloma patients that were able to lyse myeloma cells. Recently, a phase I/II clinical trial has been initiated to examine the safety and efficacy of Sp17-pulsed DC vaccination in myeloma patients 67. However, there is evidence that Sp17 is also expressed on normal T and B cells 73; hence, although these antigens may be potential targets, further research is warranted to examine their applicability for immunotherapy in MM.

T CELL-BASED IMMUNOTHERAPY IN MM

Idiotypic-based protein vaccines for myeloma

Our group at the Karolinska Institute, Stockholm, Sweden, was the first to introduce active immunization of myeloma patients with Id proteins 74,75. Considering that immunotherapy may work better in immunocompetent patients with a low tumor burden, we targeted untreated

patients with early disease. In our first pilot study, we recruited and immunized five previously untreated patients with stages I–III MM with the autologous Id protein precipitated in an aluminum phosphate suspension ⁷⁴. In three patients, an anti-Id T-cell response, detected by enumeration of IFN- γ - and IL-2-secreting cells by ELISPOT assay, was amplified 1.9- to 5-fold during the immunization. The number of B cells secreting anti-Id antibodies also increased in these three patients, and two out of the three patients had a gradual decrease of blood CD19⁺ B cells. However, the induced T-cell response was transient and was eliminated during repeated immunization. The disease was stable in all patients, and no side effects or clinical response were noted. In our second series of the study, immunization was performed by subcutaneous or intradermal injection of Id protein and granulocyte-monocyte colony-stimulating factor (GM-CSF) ⁷⁵. Five patients with IgG myeloma were treated, and an Id-specific type-1 T-cell response developed in all of them. The response involved both CD8⁺ and CD4⁺ subsets and was mainly MHC class I-restricted. There was a transient rise in B cells producing IgM anti-idiotypic antibodies in all patients. One patient had a clinical response, defined by a significant decrease in serum Id protein (from 20 g/L to 7 g/L) and normalization of serum Ig levels, which lasted for more than 1 year after immunization was started. Although these studies involved a limited number of patients, the results clearly indicated that Id protein vaccination, particularly in combination with GM-CSF, was able to induce specific anti-Id cellular and humoral immune responses, which were occasionally accompanied by a clinical response in treated patients. Furthermore, idiotype vaccination combined with IL-12 also was efficient at inducing myeloma-specific immune responses in myeloma patients ⁷⁶.

Other clinical settings for immunotherapy could be minimal residual disease status achieved by high-dose chemotherapy and early host immunologic recovery following stem cell transplantation. These are supported by a study from Massaia and coworkers ⁷⁷ showing that Id vaccination of myeloma patients with minimal residual disease was able to induce a strong Id-specific cellular immunity in many of the patients. In their study, 12 patients who had been treated with high-dose chemotherapy followed by stem cell support received Id–keyhole limpet hemocyanin (KLH) vaccines and a low dose of GM-CSF or IL-2. In most of the patients, the interval between the completion of prior high-dose therapy and vaccination was only 2 to 3 months. Generation of Id-specific T-cell proliferative responses was documented in only two cases; however, a positive, Id-specific, delayed-type hypersensitivity (DTH) skin test reaction was observed in 8 out of the 10 patients studied. The induction of humoral and cellular immune responses to KLH was observed in 100% and 80% of the patients, respectively, suggesting that the majority of patients were already able to mount immune responses to KLH shortly after high-dose therapy and stem cell transplantation. Collectively, these results indicate that immunization of myeloma patients with the autologous Id protein, together with GM-CSF, might be a promising method of immunotherapy ⁷⁸.

Dendritic cell–based vaccines for myeloma

Preclinical studies have shown that DCs generated from myeloma patients were functional and could efficiently present Id determinants to autologous T cells ^{79,80}. Compared with their progenitor monocytes, Id-pulsed DCs induced not only a stronger Id-specific T-cell response but also a predominant type-1 (IFN- γ) T-cell response ⁷⁹. Both type-1 and type-2 (IFN- γ and IL-4) T-cell responses were noted when monocytes were used as the APCs. These results indicate that DCs pulsed with Id protein can be used to induce the type-1 anti-Id response in myeloma patients.

Wen and coworkers ⁸¹ reported vaccinating a MM patient with autologous Id protein–pulsed DCs generated from blood adherent cells. Enhanced Id-specific cellular and humoral responses were observed in the patient. The immune responses were associated with a transient minor decrease in the serum Id protein level. In their subsequent study, six additional patients were

treated according to the same protocol ⁸². An immune response against Id was demonstrated in many of the patients. A minor clinical response (25% reduction in the M-component) was observed in one patient and stable disease in the remaining patients. Reichardt and coworkers ⁸³ reported their experience with Id-pulsed DC vaccination in 12 myeloma patients after autologous peripheral blood stem cell transplantation. Their results were less compelling because only 2 out of 12 patients mounted cellular Id-specific proliferative responses as the sole evidence for effective vaccination. Nevertheless, all myeloma patients could mount a strong anti-KLH response despite recent high-dose therapy. Similar results were also obtained in their subsequent study involving 26 patients treated on the same protocol ⁸⁴. Although 24 out of 26 patients generated a KLH-specific cellular proliferative immune response, an Id-specific proliferative immune response developed in only four patients. No clinical benefit was observed. These results suggest that DC-based Id vaccination is feasible after transplantation and can induce an Id-specific T-cell response in certain patients.

Other clinical trials of Id-pulsed DC vaccination in myeloma patients have been reported. Cull and coworkers ⁸⁵ reported on their experience of vaccinating two patients with advanced refractory MM with Id-pulsed DCs combined with GM-CSF. An anti-Id T-cell proliferative response was detected in both patients, which was associated with IFN- γ production by the T cells. One patient also had an anti-Id humoral response. Titzer and coworkers ⁸⁶ treated 11 patients with advanced MM with Id-pulsed, CD34⁺ stem cell-derived DCs and GM-CSF. After vaccination, 3 out of 10 analyzed patients showed an increased anti-Id antibody titer, and 4 out of the 10 patients had an Id-specific T-cell response measured by ELISPOT assay.

To improve the efficacy of DC vaccination in myeloma, we investigated the use of Id-pulsed mature DCs administered subcutaneously. In our study, five patients with stable partial remission following high-dose chemotherapy were vaccinated at least 4 months post-transplantation ⁸⁷. After four DC vaccinations, Id-specific T-cell responses, detected by ELISPOT assays (four patients) and proliferation assays (two patients), were elicited in four patients and anti-Id B-cell responses in all five patients. The cytokine-secretion profile of activated T cells demonstrated a type-1 T-cell response. A 50% reduction in serum Id protein was observed in one immunologically responding patient and persisted for more than 1 year; stable disease was noted in the other three patients. The remaining patient without an immune response to the vaccination experienced disease relapse. Similar results were recently reported by Curti and coworkers ⁸⁸. In their study, 15 patients received DCs pulsed with Id proteins or their peptides, and an Id-specific IFN- γ response was seen in 8 patients. Clinically, 7 out of the 15 patients had stable disease after a median follow-up of 26 months, one patient achieved durable partial remission after 40 months, and seven patients progressed. Alternatively, Id-pulsed allogeneic DCs could also be used to vaccinate myeloma patients ⁸⁹. Taken together, these results indicate that subcutaneous DC vaccination indeed induces better antimyeloma responses than intravenous DC vaccination.

DC vaccines can also be made in the form of fusion of tumor cells with DCs. The heterokaryons generated by tumor-DC fusion cells combine the machinery needed for immune stimulation with presentation of a large repertoire of antigens. Vaccination with fusions of tumor cells and DCs is an effective treatment in animal tumor models ^{90,91} and possibly in patients with metastatic renal carcinoma ⁹². In a murine plasmacytoma model, vaccination with DCs fused with mouse 4TOO plasmacytoma cells was associated with induction of antitumor humoral and CTL responses ⁴⁴. Immunization with the fusion cells protected mice against tumor challenge and extended the survival of tumor-established mice without eradication of the tumor cells. Addition of IL-12 helped eradicate the established tumor. In a more recent study, human myeloma cells, either primary myeloma cells from patients or a myeloma cell line, U266, were fused to human DCs ⁹³. Fusions with mature rather than immature DCs induced higher levels of T-cell proliferation and activation, as assessed by intracellular IFN- γ expression, and

stronger CTL activity against the tumor cells. Similar results were also obtained by other investigators^{94,95}. Based on these results, a clinical trial was designed to evaluate the efficacy of vaccinating myeloma patients with a fusion of myeloma cells and autologous mature DCs⁹³.

DNA vaccines for myeloma

Various approaches to antitumor therapy that use both antigen-encoding DNA and noncoding nucleotides as components of genetic vaccination are currently being explored⁹⁶. These strategies include the construct that fuses an scFv incorporating both variable-region genes necessary to encode the Id determinants with fragment C of tetanus toxin²⁸ and gene transfer of cytokines or costimulatory molecules into myeloma cells by nonviral and viral vectors⁹⁷. In animal studies, DNA vaccination promoted specific immune responses and induced strong protection against B-cell lymphoma and myeloma^{28,98}. These strategies may have implications for immunotherapy in human diseases.

A phase I study has been completed to evaluate the feasibility and safety of vaccinating MM patients after high-dose chemotherapy with adenovector-engineered, IL-2-expressing autologous plasma cells⁹⁹. Eight patients were enrolled and vaccines were successfully made in six patients, who received one to five subcutaneous injections of $3.5\text{--}9.0 \times 10^7$ cells/injection. Vaccines were well tolerated, with only minor systemic symptoms reported. Vaccination induced a local inflammatory response consisting predominantly of CD8⁺ T cells. However, no specific antitumor immune or clinical responses were noted. Hence, further studies of immunological and clinical efficacy are needed to examine the applicability of this approach to the treatment of patients.

Combined donor vaccination and allogeneic stem-cell transplantation

Allogeneic stem cell transplantation showed efficacy in MM but was accompanied by severe GVHD^{100–103}. One strategy for enhancing antimyeloma effects without aggravating GVHD is to target an immune response selectively against a defined tumor-specific antigen. This could be accomplished by eliciting an antimyeloma immune response in allogeneic HSCT donors by active immunization prior to allogeneic HSCT and/or DLI. This strategy was pioneered by Kwak and coworkers in 1995¹⁰⁴. An HLA-matched donor received 2 subcutaneous immunizations of patient-derived idiotype conjugated to KLH at a one-week interval before marrow harvest. The recipient patient demonstrated no pre-existing anti-idiotypic immunity pre-transplantation. Thirty and 60 days after conditioning with busulfan and cyclophosphamide and transfer of unmanipulated donor bone marrow, significant lymphocyte proliferative responses against the idiotype were detected in the recipient. A CD4⁺, idiotype-specific T-cell line was generated from the recipient's blood, which was, unequivocally, of donor origin because in situ hybridization assay demonstrated the presence of Y chromosome in more than 95% of the T cells. By day 220, a greater than 90% reduction in serum M-protein was observed, which persisted for over 3 years.

Based on this encouraging result from the single patient mentioned above, the investigators, then at the National Cancer Institute (NCI), opened a clinical trial of donor immunization in MM under an FDA-approved Investigational New Drug application in collaboration with the Arkansas myeloma research group at the University of Arkansas. The clinical protocol was designed to explore whether a booster immunization of the recipient might improve the potency and duration of the transferred idiotype-specific response. Five additional donor-recipient pairs were enrolled and vaccinated with idiotype-KLH protein plus GM-CSF. Two recipients succumbed to early post-allogeneic stem cell transplantation complications, unrelated to vaccination. The 3 remaining recipients achieved and remained in continuous complete remission 3.5, 4, and 5 years after transplantation. One recipient suffered from chronic GVHD

and was on chronic steroid therapy, while the other 2 recipients and all of the donors were medically well, without any significant complications. In all 3 recipients, transfer of T-cell responses to the KLH carrier protein has been documented. Analysis and serial monitoring of idiotype-specific T-cell responses in the donors and recipients have been in progress ¹⁰⁵.

Taken together, these proof-of-principle studies demonstrate a direct transfer of myeloma idiotype-specific T-cell immunity from donor to recipient following allogeneic stem cell transplantation or donor lymphocyte infusion. These results also suggest that the donor-derived T-cell response was not blocked by circulating myeloma idiotype protein in recipient during and after transplantation, or inhibited by the immunosuppressive medication used for GVHD prophylaxis in the patients. Furthermore, GVHD did not appear to be exacerbated secondary to this immunotherapeutic maneuver.

Immunotherapy with donor-derived or patient-derived, tumor-specific lymphocytes

Successful immunotherapy of patients with tumors requires the *in vivo* generation of large numbers of highly reactive antitumor lymphocytes that are not restrained by normal tolerance mechanisms and are capable of sustaining immunity against tumor cells. Immunizing patients with MM with myeloma antigens such as the idiotype proteins or tumor lysate can increase the number of circulating antigen-specific T cells. To date this has not correlated with clinical tumor regression, suggesting that the numbers of these T cells, particularly those of the CTLs, are still insufficient to cause major tumor damage, and/or there are defects in function or activation of these T cells.

Falkenburg and coworkers reported the first successful treatment of a hematological malignancy with donor-derived tumor-specific CTL lines in 1999 ¹⁰⁶. A patient with accelerated phase chronic myeloid leukemia (CML) received infusions of 3 donor-derived leukemia-reactive CTL lines at 5-week intervals at a cumulative dose of 3.2×10^9 CTLs after allogeneic HSCT. The CTLs were selected based on their ability to inhibit the *in vitro* growth of CML progenitor cells and to lyse the leukemic cells from the patient. Shortly after the third infusion, complete eradication of the leukemic cells was observed, as shown by cytogenetic analysis, fluorescence in situ hybridization, molecular analysis of BCR/ABL mRNA, and chimerism studies. Thus, these results show that *in vitro* cultured leukemia-reactive CTLs can be successfully applied to treat accelerated phase CML after allogeneic HSCT.

In addition to obtaining tumor-specific CTLs from donors, these cells can also be obtained from patients themselves, *ex vivo* expanded, and used adoptively to eradicate tumor cells. In patients with metastatic melanoma refractory to treatment with high dose IL-2 and to chemotherapy, the transfer of *in vitro*-activated and expanded autologous antitumor lymphocytes plus IL-2 into lymphodepleted patients mediated objective cancer regression in 6 out of 13 patients. Persistence of the transferred cells was seen for up to 4 months after cell administration ¹⁰⁷. The number of patients enrolled on this protocol was expanded and the investigators have observed objective cancer regression in 18 out of 35 patients (51%), many of whom have bulky disease ¹⁰⁸. These studies demonstrate that adoptive transfer of tumor-reactive lymphocytes after nonmyeloablative conditioning can be an effective treatment for patients with metastatic cancers.

ANTIBODY-BASED IMMUNOTHERAPY IN MM

Within the past decade, mAbs have broadened the therapeutic armamentarium in oncology ¹⁰⁹. Hematological malignancies are recognized as particularly promising targets, reflected by the current list of FDA-approved mAbs that are used to treat patients ^{110,111}. The mAbs exert their *in vivo* effect largely through the immunological effector mechanisms of complement-mediated lysis (CDC) and/or antibody-dependent cell-mediated cytotoxicity

(ADCC). Thus, their efficacy depends on intact immunological mechanisms in the treated patients. Although the molecules targeted by these mAbs are usually widely expressed on normal lymphoid and myeloid cells in addition to malignant cells, the therapeutic efficacy of these mAbs has been promising^{109–111}. Nevertheless, it will be useful to develop mAbs with an inherent capability to kill tumor cells, that is, independent of complement and ADCC, and with selectivity toward neoplastic cells.

Targeting surface β_2 -microglobulin to induce apoptosis in myeloma cells

β_2 -microglobulin (β_2 M) is an 11.6-kDa non-glycosylated polypeptide composed of 100 amino acids. It is part of the MHC class I molecule on the cell surface of nucleated cells. Its best characterized function is to interact with and stabilize the tertiary structure of the MHC class I α -chain¹¹². Because it is non-covalently associated with the α -chain and has no direct attachment to the cell membrane, β_2 M on the cell surface can exchange with free β_2 M present in serum-containing medium¹¹³. Free β_2 M is found in body fluids under physiological conditions as a result of intracellular release. Elevated levels of serum β_2 M are present in hematological malignancies, including lymphomas¹¹⁴, leukemias^{115,116}, and MM^{2,117} and correlate with a poor prognosis regardless of a patient's renal function^{117,118}. This observation suggests an important, yet unidentified, role of this protein in these malignancies. While examining the effects of β_2 M on myeloma cells, we made a novel and exciting discovery, namely that mAbs against β_2 M have a remarkably strong apoptotic effect on myeloma cells and on other hematological tumor cells¹¹⁹. Anti- β_2 M mAbs induced apoptosis in up to 90% of cells in a 48-hour culture in all tested human myeloma cell lines ($n = 8$) and primary myeloma cells from patients ($n = 10$). The mAbs also kill β_2 M/MHC class I-bearing lymphoma and leukemia cells. Anti-MHC class I mAbs (LY5.1, IgG1 or W6/32, IgG2a), purified mouse IgG and IgG1 had no effect. Cell death occurred rapidly, without the need for exogenous immunological effector mechanisms (e.g., complement or NK cells) or secondary cross-linking. Anti- β_2 M mAb-induced apoptosis in myeloma cells were not blocked by soluble β_2 M (10–100 μ g/mL, 3- to 30-fold higher than the levels in most MM patients), IL-6, or other myeloma growth and survival factors and was stronger than apoptosis observed with chemotherapy drugs currently used to treat MM (e.g., dexamethasone).

Although the expression of β_2 M on normal hematopoietic cells is a potential safety concern, the mAbs were selective to tumor-transformed cells and did not induce apoptosis of normal cells, including T and B lymphocytes, plasma cells, and purified CD34⁺ stem cells. Furthermore, the mAbs selectively and effectively killed myeloma cells without damaging osteoclasts (OCs) or PBMCs in their cocultures with myeloma cells. More importantly, anti- β_2 M mAbs are therapeutic in vivo in xenograft SCID (Figure 2) and SCID-hu mouse models¹¹⁹, and in the HLA-A2-transgenic NOD-SCID (A2-NOD-SCID) models of myeloma, in which every mouse tissue expresses human MHC class I/ β_2 M molecules and circulating human β_2 M could reach the levels seen in most myeloma patients without causing damage to normal human hematopoiesis or murine organs¹²⁰. Interestingly, following our publication, others have reported similar results using anti-MHC class single-chain Fv diabody or anti- β_2 M antibodies, respectively, in human myeloma¹²¹, renal cell carcinoma¹²², and prostate cancer¹²³. Therefore, such mAbs offer the potential for a therapeutic approach to hematological malignancies.

The mAbs induced apoptosis in myeloma cells by recruiting MHC class I to lipid rafts, activated JNK, and inhibited PI3K/Akt and ERK pathways¹¹⁹. Growth and survival cytokines such as IL-6 and IGF-I, which could protect myeloma cells from dexamethasone-induced apoptosis, did not affect mAb-mediated cell death. We elucidated the mechanisms underlying anti- β_2 M mAb-induced PI3K/Akt and ERK inhibition and the inability of IL-6 and IGF-I to protect myeloma cells from mAb-induced apoptosis. We focused on lipid rafts and confirmed that

these membrane microdomains are required for IL-6 and IGF-I signaling. By recruiting MHC class I into lipid rafts, anti- β_2 M mAbs excluded IL-6 and IGF-I receptors and their substrates from the rafts. The mAbs were not only redistributed the receptors in cell membrane, but also abrogated IL-6- or IGF-I-mediated JAK/STAT3, PI3K/Akt, and Ras/Raf/ERK pathway signaling, which are otherwise constitutively activated in myeloma cells ¹²⁴. Thus, our study further defines the tumoricidal mechanism of the mAbs and provides strong evidence to support the potential of these mAbs as therapeutic agents for myeloma.

Anti-CS1 antibodies

CS1, a glycoprotein and a member of the immunoglobulin gene superfamily, has been found to be highly expressed on tumor cells from myeloma patients, and soluble serum CS1 correlates with active disease in myeloma patients ¹²⁵. However, CS1 is also expressed by NK cells, NKT cells, and CD8⁺ T cells ¹²⁵. Recent studies demonstrated that CS1 promotes myeloma cell adhesion, clonogenic growth, and tumorigenicity via c-maf-mediated interactions with bone marrow stromal cells ¹²⁶.

As the above data suggest that CS1 could be a novel target for therapy, a humanized mAb against CS1, HuLuc63, was generated ¹²⁵. HuLuc63 inhibited myeloma cell binding to bone marrow stromal cells and induced ADCC against myeloma cells in dose-dependent and CS1-specific manners. Furthermore, the mAb mediated autologous ADCC against primary myeloma cells resistant to conventional or novel therapies, and pretreatment with conventional or novel antimyeloma drugs markedly enhanced HuLuc63-induced myeloma cell lysis. In vivo injection of the mAb significantly induced tumor regression in xenograft myeloma mouse models ¹²⁷. Based on these results, phase I clinical trials are underway to evaluate the safety and toxicity of the mAb in myeloma patients.

Other antibodies

Inhibiting DKK1 activity by using specific mAbs to treat MM and myeloma-associated bone disease is a novel approach because DKK1 has been shown to contribute to osteolytic bone disease in MM by inhibiting the differentiation of osteoblasts ⁵². A humanized DKK1-neutralizing mAb, BHQ880 has been developed by Novartis and tested in preclinical studies ^{53–55}. In both murine ⁵⁴ and xenograft human ^{53,55} myeloma mouse models, this mAb was shown to sustain or increase the numbers of osteoblasts, protect myeloma-induced bone loss, and reduce the development of osteolytic bone lesions. Furthermore, the mAb was also shown to inhibit the growth of xenografted human myeloma cells in SCID-hu ⁵⁵ or SCID-rab ⁵³ mouse models. These results provide the rationale for clinical evaluation of BHQ880 to improve bone disease and to inhibit myeloma growth.

Another potential target is CD40, which is expressed on B-cell tumors including MM. Two humanized anti-CD40 mAbs, SGN-40 and HCD122, have been developed and tested in preclinical studies ^{128,129}. These mAb induced modest cytotoxicity in myeloma cell lines and primary myeloma cells from patients, but can effectively kill myeloma cell via mediating ADCC. Further, the immunomodulatory drug lenalidomide further augmented anti-CD40 mAb-induced cytotoxicity in human myeloma cells ¹³⁰. In addition to anti-CD40 mAbs, other mAbs currently in clinical trials include anti-CD74, anti-CD56, and anti-HM1.24 ¹³¹.

CONCLUSION

Various clinical immunotherapy treatment strategies have been tested in B-cell malignancies, including MM. Most of these have focused on targeting idiotypic-specific immunity. Idiotypic-based vaccines have been shown to induce or enhance idiotypic-specific immunity, indicating that the vaccines are able to elicit a specific immune response. However, clinical response is

still a rare event, occurring only in a minority of treated patients, suggesting that the elicited or enhanced immunity is still too weak to cause significant tumor destruction. Alternatively, a non-beneficial immune response (such as the type-2 T-cell response) may also be generated by immunization, which may enhance tumor B-cell growth and facilitate differentiation into plasma cell tumors.

Ideally, a tumor-specific immunotherapy should induce or expand only the beneficial immune responses mediated by CTLs (Th1 and Tc1 subsets) that have sufficient cytotoxic effects toward tumor cells but not normal cells. Further studies are warranted so that we can better understand the immune regulation mechanism in MM.

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Figure 1. Cytotoxicity of DKK1 peptide-specific T-cell clones

P20-specific or P66-specific T-cell clones against human myeloma cell lines U266 (DKK1⁺/HLA-A*0201⁺) and ARP-1 (DKK1⁺/HLA-A*0201⁻), and primary myeloma cells from four patients with MM (MM1 to MM4). Control target cells include normal mesenchymal stem cells (MSCs), DCs, B cells, and PBMCs from HLA-A*0201⁺ healthy donors. Patients #1 and #2 were HLA-A*0201⁺, and patients #3 and #4 were HLA-A*0201⁻. All primary myeloma cells expressed DKK1 protein. P20 and P66 are two DKK1 peptides that have high affinity for HLA-A*0201. An effector:target (E:T) ratio of 10:1 was used. Results of four independent experiments with two CTL clones generated from a patient with MM are shown.

Figure 2.

In vivo therapeutic effects of β_2 M-specific mAbs on established human myeloma in a SCID mouse model. Mice were xenografted subcutaneously with ARP-1, and tumor burdens were monitored as tumor volumes. Mice received intraperitoneal injections every 3 days for a total of 4 injections of 250 μ l ascites containing about 500 μ g D1 β_2 M-specific mAb or 500 μ g mouse IgG1, or an equal volume of PBS. Results (image of tumor burdens in the mice) from one representative experiment out of three performed using D1 mAb with 5 mice per group are shown.