Dickkopf-1 (Dkk1) is a widely expressed and potent tumor-associated antigen in multiple myeloma

Jianfei Qian,1 Jin Xie,2 Sungyoul Hong,1 Jing Yang,1 Liang Zhang,1 Xiaohong Han,1 Michael Wang,1 Fenghuang Zhan,2 John D. Shaughnessy Jr,2 Joshua Epstein,2 Larry W. Kwak,1 and Qing Yi1

1Department of Lymphoma and Myeloma, Division of Cancer Medicine, and the Center for Cancer Immunology Research, The University of Texas M. D. Anderson Cancer Center, Houston, TX; and 2Myeloma Institute for Research and Therapy, Arkansas Cancer Research Center, University of Arkansas for Medical Sciences, Little Rock

The identification of novel tumor-associated antigens, especially those shared among patients, is urgently needed to improve the efficacy of immunotherapy for multiple myeloma (MM). In this study, we examined whether Dickkopf-1 (Dkk1), a protein that is not expressed in most normal tissues but is expressed by tumor cells from almost all patients with myeloma, could be a good candidate. We identified and synthesized Dkk1 peptides for human leukocyte antigen (HLA)–A*0201 and confirmed their immunogenicity by in vivo immunization in HLA-A*0201 transgenic mice. We detected, using peptide-tetramers, low frequencies of Dkk1 peptide-specific CD8-positive (CD8+) T cells in patients with myeloma and generated peptide-specific T-cell lines and clones from HLA-A*0201-positive (HLA-A*0201+) blood donors and patients with myeloma. These T cells efficiently lysed peptide-pulsed but not unpulsed T2 or autologous dendritic cells, Dkk1-positive (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-negative (HLA-A*0201-) myeloma cell lines and primary myeloma cells or HLA-A*0201+ normal lymphocytes, including B cells. These results indicate that these T cells were potent cytotoxic T cells and recognized Dkk1 peptides naturally presented by myeloma cells in the context of HLA-A*0201 molecules. Hence, our study identifies Dkk1 as a potentially important antigen for immunotherapy in MM. (Blood. 2007;110:1587-1594)

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Introduction

Multiple myeloma (MM) is still a fatal hematological malignancy characterized by the accumulation of terminally differentiated plasma cells in the bone marrow of patients.1 The outcome of the majority of patients with MM is unsatisfactory, although they benefit from high-dose therapy followed by autologous stem-cell support.2 There is a clear need for new treatments to stabilize or even eradicate minimal residual disease achieved after the treatment with high-dose chemotherapy and stem-cell transplantation. Immunotherapy may be an appropriate means to control residual disease as well as to provide an alternative treatment modality to conventional chemotherapy for patients with MM.

The demonstration of autologous idiotype-specific T cells3 and evidence of clinical response to allogeneic donor lymphocyte infusions1,4,6 indicate that antimyeloma responses can be generated. Specific cytotoxic T lymphocyte (CTL)–mediated immunotherapy for MM can be achieved by vaccination using the idiotype proteins isolated from the serum of patients.7 However, the idiotype proteins represent a unique myeloma-associated antigen and thus cannot provide shared immunotherapy for various patients with MM. Although immunotherapy in combination with high-dose chemotherapy holds great promise for the treatment of MM, clinical studies have not yet delivered the expected results. In addition to optimizing immunotherapy methods, there is an urgent need to search for and use novel shared myeloma antigens to efficiently stimulate antimyeloma CTL responses in most treated patients.

Dickkopf-1 (Dkk1) is a secreted protein that specifically inhibits Wnt/β-catenin signaling by interacting with the coreceptor Lrp-6.8,9 Previous studies have shown that the Dkk1 gene has a restricted expression in placenta and mesenchymal stem cells (MSCs) only, not in other normal tissues.10,11 Recent studies have demonstrated that Dkk1 in patients with myeloma is associated with the presence of lytic bone lesions.12 Immunohistochemical analysis of bone marrow biopsy specimens shows that only myeloma cells contain detectable Dkk1. Recombinant human Dkk1 or bone marrow serum containing an elevated level of Dkk1 inhibits the differentiation of osteoblast precursor cells in vitro. Furthermore, anti-Dkk1 antibody treatment is associated with reduced tumor growth in a myeloma mouse model. These results indicate that Dkk1 is an important player in myeloma bone disease and blocking Dkk1 activity reduced osteolytic bone resorption, increased bone formation, and helped control myeloma progression.13

The unique properties and restricted expression profile of Dkk1 led us to hypothesize that it might be a potent tumor-associated antigen (TAA) in MM. In the present study, we reevaluated Dkk1 expression profile, searched for high-affinity Dkk1 peptides for HLA-A*0201, examined the immunogenicity of Dkk1 peptides using peptide immunization in HLA-A*0201 transgenic mice, and generated and characterized Dkk1 peptide-specific CTLs from healthy blood donors and patients with

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myeloma. Our study identifies DKK1 as a potentially important antigenic target for antmyeloma immunotherapy.

Materials and methods

This study was approved by the Institutional Review Board Committee of The University of Texas M. D. Anderson Cancer Center, and informed consent was obtained in accordance with the Declaration of Helsinki.

Myeloma cells

Human myeloma cell lines (HMCLs) used include U266, IM-9, XG1, AR1, ARK, JHMT, MM1-144, H929, OPM1, OPM2, KMS18, and MM1S. All cell lines were maintained in RPMI 1640 medium (Thermo Fisher Scientific, Waltham, MA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA). K562 (American Type Culture Collection, Manassas, VA) were used as natural killer (NK) cell–sensitive targets. Primary myeloma cells were isolated from bone marrow aspirates from patients with myeloma with the use of density centrifugation and anti-human CD138 antibody-coated magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany).14 Aliquots of purified myeloma cells were cryopreserved in liquid nitrogen until use.

Generation of dendritic cells

Monocyte-derived mature dendritic cells (DCs) were generated from peripheral blood mononuclear cells (PBMCs) using standard protocol.14-17 In brief, PBMCs were allowed to adhere in culture flasks for 2 hours, and nonadherent cells were collected and cryopreserved for future use. The adherent cells were cultured in Aim-V medium (Invitrogen, Carlsbad, CA) supplemented with granulocyte macrophage-colony-stimulating factor (GM-CSF) (10 ng/mL) and interleukin (IL)-4; 10 ng/mL; both from R&D Systems, Minneapolis, MN), with further addition of cytokines every other day. After 5 days of culture, DCs were induced to maturation by addition of tumor necrosis factor-α (10 ng/mL) and IL-1β (10 ng/mL; both from R&D Systems) for 48 hours.

Immunophenotyping and intracellular cytokine staining

Phycoerythrin- or fluorescein isothiocyanate (FITC)–conjugated monoclonal antibodies (mAbs) were added to cell pellets, incubated for 30 minutes on ice, and washed 3 times before analysis. Intracellular cytokine staining was performed using the Cytotox/Cytoperm kit (BD Biosciences, San Jose, CA) according to the manufacturer’s recommendations. Samples were analyzed using a flow cytometer (FACSCalibur; BD Biosciences).

Peptide-T2 cell binding assay

All peptides were synthesized in the Peptide Synthesis Facility at the M. D. Anderson Cancer Center. Purity of synthetic peptides was confirmed, with the use of reversed-phase high-performance liquid chromatography and mass spectrometry, to be more than 98%. Synthetic peptides were analyzed using a flow cytometer (FACSCalibur; BD Biosciences).

Determination of in vivo immunogenicity of the peptides

HLA-A*0201 transgenic (Tg[HLA-A2.1]) mice15-21 were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained at the animal facility and studies were approved by the Institutional Animal Care and Use Committees of The University of Texas M. D. Anderson Cancer Center.

For immunization, peptides were diluted in phosphate-buffered saline at room temperature, mixed, and emulsified with an equal volume of incomplete Freund’s adjuvant (Sigma). Groups of 3 mice were immunized subcutaneously at the tail base with 100 μL of peptide emulsion containing 100 μg of peptides. Two weeks after the immunization, mice were killed and splenocytes were isolated for in vitro studies.

Reverse transcriptase–polymerase chain reaction for detecting DKK1 mRNA expression

Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using a PTC-1000 programmable thermal controller (MJ Research, Waltham, MA) with QuantiTect SYBR Green PCR kit (QIAGEN, Valencia, CA) according to the manufacturer’s instructions. RNA was isolated from tumor cells with an RNeasy Mini kit (QIAGEN), and human total RNA master multipurpose panel from Clontech (Mountain View, CA) were used. Random-primed cDNA synthesis was performed using Quantitect Reverse Transcription Kit (QIAGEN). For amplifications, the following primers were designed: DKK1: forward, 5'-AGA CCA TGA ACA ACT ACC AGC CGT-3'; reverse, TCT GGA ATA CCC ATC CAA GGT GCT GCT-3'; and GAPDH: forward, 5'-CCT CCG GGA AAA TGT GGC GTG ATG G-3'; reverse, 5'-AGA CCG CAG GTC AGG TCC ACC ACC GTG-3'. Each of the primer sets was confirmed by running samples on agarose gels. GAPDH transcript levels were used to normalize the amount of cDNA in each sample.

Western blot analysis

Western blot analysis was used to detect DKK1 protein expression in myeloma cells. Cell lysates were prepared from purified primary myeloma cells and cell lines and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. After transfer to nitrocellulose membrane and subsequent blocking, the membranes were immunoblotted with goat anti-human DKK1 antibody (R&D Systems) and visualized with horseradish peroxidase (HRP)–conjugated donkey anti–goat IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA), followed by enhanced chemiluminescence (Pierce Biotechnology, Rockford, IL) and autoradiography.

Generation of DKK1-specific T-cell lines and clones

DKK1-specific T cells were generated from PBMCs of HLA-A*0201–positive (HLA-A*0201+) blood donors and patients with myeloma by repeated stimulation of autologous T cells with DKK1 peptide-loaded mature DCs. In brief, the nonadherent cells of PBMCs (2 × 10^6/mL; used as T-cell population) were cocultured in 50-mL tissue-culture flasks at 37°C in 5% CO₂ for 7 to 10 days in Aim-V medium supplemented with 10% pooled human serum (T-cell medium) with mature DCs (2 × 10^6/mL) preincubated with DKK1 peptides at a final concentration of 50 μg/mL at 37°C for 2 hours. After culture, T cells were collected and restimulated with DKK1 peptide-pulsed autologous mature DCs every week, and the cultures were fed every 5 days with fresh medium containing recombinant IL-2 (20 IU/mL), IL-7 (5 ng/mL), and IL-15 (5 ng/mL; all from R&D Systems). Induction of DKK1-specific T cells was monitored weekly using T-cell proliferation assay and DKK1 peptide-HLA-A*0201 tetramers (synthesized by MHC Tetramer Laboratory, Baylor College of Medicine, Houston, TX). After 3 to 4 cycles of in vitro stimulation and selection, T-cell lines were established, and T-cell clones were obtained by limiting-dilution assay. T-cell lines and clones were expanded in T-cell medium containing IL-2 (100 IU/mL), IL-7 (5 ng/mL), and IL-15 (5 ng/mL) for 2 weeks and subjected to functional tests.

Proliferation assays

T cells (5 × 10^4/100 μL/well) were seeded into 96-well U-bottomed tissue culture plates (Corning Glassworks, Corning, NY) in T-cell medium. Various numbers of autologous mature DCs loaded with or without DKK1 peptides were added to the plates and cultured for 4 days at 37°C in 5% CO₂. T-cell proliferation was measured after overnight incubation with
DKK1 was a widely expressed in myeloma but not normal cells

RT-PCR and Western blot analyses were used to examine the expression of DKK1 in normal human tissues and myeloma cells. As shown in Figure 1A, DKK1 mRNA was not detected in most human tissues except prostate, testis, placenta, and uterus. DKK1 mRNA was detected in all 8 myeloma cell lines and primary myeloma cells from 10 patients examined, and in MSCs but not PBMCs from a healthy blood donor (Figure 1B). Likewise, DKK1 protein was detected in 7 of 10 myeloma cell lines and in all primary myeloma cells from 10 patients with MM (Figure 1C).

Selection of DKK1 peptides

The sequence of DKK1 was reviewed for peptides that could potentially bind to HLA-A*0201 using a peptide binding database (http://www-bimas.cit.nih.gov/molbio/hla_bind/). After comparing the predictive binding scores, we identified and selected 2 peptides that could potentially bind with HLA-A*0201 molecules: one with the highest binding score (P20) and one with a low score (P66) (Table 1). Heteroclitic peptides were made by replacing position-1 amino acid with tyrosine (Py20) or C-terminal amino acid with valine (P66v)25,26; these had significantly higher predictive binding scores. We selected the unmodified P20 and heteroclitic P66v for the following experiments on the basis of the binding affinity (Figure 2A) and stability (Figure 2B) measured by peptide-T2 binding assay. Peptides from influenza virus matrix protein (Flu-matrix),27 and HIV type 1 reverse transcriptase (HIV-pol)28 were used as controls.

In vivo immunogenicity of synthetic DKK1 peptides

To examine whether the peptides were able to immunize HLA-A*0201 transgenic mice, we subcutaneously injected 100 μg per mouse of peptides P20 and P66v, and Flu-matrix peptide as positive control to the mice (3 mice per peptide) according to established protocol.29-31 Two weeks after the immunization, mice were killed, and splenocytes were collected, restimulated with the immunizing peptides for 5 days, and subjected to analyses. In vivo immunization successfully generated peptide-specific T cells, detected as specific IFN-γ-expressing (Figure 2C) and peptide (P20 or P66v; Figure 2D)-HLA-A*0201+ CD8-positive (CD8+) T cells.

Table 1. Potential DKK1 and control peptides for HLA-A*0201 molecules

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Position</th>
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<td>66</td>
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<td>58</td>
<td>550</td>
<td>1.55</td>
</tr>
<tr>
<td>HIV pol</td>
<td>ILKEPVNQG</td>
<td>476</td>
<td>39</td>
<td>1.26</td>
</tr>
</tbody>
</table>

DKK1 indicates Dickkopf-1; HLA, human leukocyte antigen.

* Estimate of half-time of dissociation and calculated score in arbitrary units.
†(Mean fluorescence with peptide-mean fluorescence without peptide)/(mean fluorescence without peptide). Results are representative of 2 experiments.

Statistical analysis

The Student t test was used to compare various experimental groups. A P value less than .05 was considered statistically significant. Unless otherwise indicated, means and standard deviations (SD) are shown.
Furthermore, the splenocytes displayed strong cytolytic activity against peptide-pulsed, but not unpulsed, murine DCs (Figure 2E). P20 and P66v appeared to be as immunogenic as the Flu-matrix peptide in immunizing the mice. These results indicate that the DKK1 peptides were able to induce a strong peptide-specific CTL response in HLA-A*0201 transgenic mice.

**Generation of DKK1 peptide-specific T-cell lines**

We first examined whether DKK1 peptide-specific CTL precursor cells were present in patients with MM. Using peptide (P20 or P66v)-HLA-A*0201 tetramers to stain T cells of patients with myeloma and healthy blood donors, we showed that DKK1 peptide-specific CD8+ T cells were detected, although at low frequencies, in 2 patients with myeloma, whereas the frequency of such T cells was much lower in the healthy blood donors (Figure 3).

To generate DKK1 peptide-specific T cells from HLA-A*0201+ blood donors and patients with myeloma, autologous mature DCs pulsed with peptides were used as APCs. After 3 to 4 rounds of in vitro stimulation, T-cell lines were obtained that proliferated in response to autologous DCs pulsed, but not unpulsed, with DKK1 peptides P20 (P < .01 compared with unpulsed DC; Figure 4A) or P66v (P < .01 compared with unpulsed DC; Figure 4B). The same results were also obtained with a CFSE-labeling assay to measure T-cell proliferation (Figure 4C). Using peptide-tetramer staining, we showed that the frequencies of peptide-specific CD8+ T cells increased during in vitro stimulation; from 3% to 4% of specific T cells at the second stimulation to 13% to 15% at fourth stimulation (Figure 4D). The standard 4-hour 51Cr-release assay was used to examine the cytotoxicity of the T cells. As exemplified by the results obtained with a T-cell line specific for P20 (Figure 4E) or P66v (Figure 4F) generated from patients with myeloma, the T cells specifically lysed autologous primary myeloma cells and DKK1-positive (DKK1+/HLA-A*0201+) U266 but not DKK1-/HLA-A*0201-negative (HLA-A*0201-) ARP-1 cells. These results suggest that the T-cell lines recognized the DKK1 peptides that are naturally processed and presented in the context of HLA-A*0201 molecules on myeloma cells.

**Cloning and characterizing DKK1 peptide-specific CTLs**

Using limiting-dilution assay, we obtained 3 T-cell clones (T4, T12, and T16; Figure 5A) from a P20-specific T-cell line and 5 T-cell clones (T6, T19, T23, T26, and T27; Figure 5B) from a P66v-specific T-cell line generated from patients with MM. These T-cell clones were identified by secretion of IFN-γ in response to antigen stimulation. After expansion and further selection, we chose clone T16 (P20-CTL) and clone T6 (P66v-CTL) for further functional studies.

We assessed the cytotoxic activity of the clones against T2 cells pulsed with DKK1 or control peptides. First, we examined the cytolytic activity of the T-cell clones against T2 cells pulsed with different concentrations of DKK1 peptides and demonstrated a dose-dependent response (Figure 5C). Second, we showed that these T-cell clones lysed T2 pulsed with the specific DKK1 peptides P20 or P66v (P < .01 compared with control peptides) but not unpulsed T2 cells or T2 cells pulsed with irrelevant DKK1 or control (Flu-matrix and HIV-pol) peptides (Figure 5D), further confirming the specificity of the T-cell clones.

Next, we examined the cytolytic activity of the T-cell clones against myeloma cells, including HMCLs and primary myeloma cells isolated from patients with MM. As shown in Figure 6A,B, the T-cell clones effectively lysed DKK1+/HLA-A*0201+ HMCLs U266 and IM-9 cells, but not DKK1-negative (DKK1-) HL A-A*0201- XG1 cells or DKK1+/HLA-A*0201+ ARP-1, and MM.1S
cells (data not shown). No killing was observed against K562 cell line, excluding the possibility that NK cells contributed to the cytotoxicity. Furthermore, the T-cell clones efficiently killed DKK1/HLA-A*0201 primary myeloma cells from patients 1 and 2 but not myeloma cells from 2 DKK1/HLA-A*0201 patients (patients 3 and 4) (Figure 6C). All together, these results demonstrate that the T-cell clones were able to lyse not only DKK1 peptide-pulsed T2 cells but also myeloma cells including primary myeloma cells from patients positive for HLA-A*0201 with MM, further confirming our findings that the DKK1 peptides are naturally presented in the context of HLA-A*0201 molecules by primary myeloma cells and shared among patients.

To determine MHC restriction of the T-cell–mediated cytotoxicity, we evaluated the inhibitory effects of anti-MHC mAbs. As shown in Figure 6D, mAbs against HLA-ABC or HLA-A*0201 significantly inhibited (70%-80% inhibition) T-cell–mediated cytotoxicity against peptide-pulsed T2 cells (P < .01 compared with medium control). No inhibitory effect was observed with mAb against HLA-DR and isotype control IgG. The results indicate that the cytotoxicity was attributed to MHC class I and, more specifically, HLA-A*0201-restricted CD8+ CTLs.

Finally, we examined whether the T cells were cytolytic to normal hematopoietic cells. In these experiments, autologous mature DCs, purified blood B cells (using anti-CD19 antibody-coated microbeads) and PBMCs, and MSCs (DKK1-expressing cells) from HLA-A*0201 subjects were used as target cells. As shown in Figure 6C, the T-cell clones did not kill DKK1+ DCs, B cells, or PBMCs but lysed DKK1+ MSCs, although the cytolytic activity against MSCs was weaker than that against myeloma cells.

**Cytotoxicity of the T cells was mediated via the perforin exocytosis pathway**

Flow cytometry analysis was used to examine the expression of granzyme, perforin and Fas ligand (FasL) by the T-cell clones. As shown in Figure 7A, it seems that the T-cell clones killed their target cells via the perforin/granzyme pathways, because they expressed high levels of perforin and granzyme B but not FasL. The T-cell clones also expressed CD45RO but not CD45RA, indicating that they were memory effector cells.

**Expression and production of IFN-γ by T cells**

Two independent methods were used to examine the cytokine expression profiles of the T cells. Figure 7B shows a representative experiment of intracellular cytokine staining for IFN-γ and IL-4 expression in the P66v-specific T-cell clone. On restimulation with...
In the present study, we demonstrated that DKK1 gene and protein were expressed in most myeloma cell lines and primary myeloma cells from all patients examined, indicating that DKK1 might be an excellent candidate as a TAA in MM. Therefore, we identified DKK1 peptides for HLA-A*0201 molecules and generated DKK1 peptide-specific CTLs from HLA-A*0201 blood donors and patients with myeloma. The CTLs had strong cytolytic activity against DKK1 peptide-pulsed T2 cells and autologous DCs, DKK1/HLA-A*0201+ HMCLs U266 and IM-9, and, more importantly, primary myeloma cells from HLA-A*0201+ patients. These CTLs did not kill normal blood cells, indicating that they may be promising effector cells for immunotherapy in MM.

In addition to myeloma cells, DKK1 mRNA is detected in some normal tissues such as testis, prostate, placenta, and uterus. Whether DKK1 protein is also expressed in these tissues remains to be examined. On the basis of the expression pattern, 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. In this study, we tested the CTLs against normal blood cells, including DCs, B cells, and PBMCs, which do not express DKK1. As expected, the CTLs did not kill these cells but lysed MSCs, because MSCs expressed DKK1 protein. However, because MSCs display immunosuppressive activity in both animals and humans, it remains to be determined whether the CTLs could kill these cells in vivo. Furthermore, some evidence indicates that chemotherapy drugs such as thalidomide and lenalidomide up-regulated DKK1 mRNA expression in myeloma cells, and dexamethasone enhanced DKK1 expression in osteoblasts. Taken together, these observations warrant further studies to examine the reactivity and impact of DKK1-specific CTLs on normal tissues that express DKK1 protein, and whether chemotherapy could further enhance the sensitivity of myeloma cells and MSCs to the CTL-mediated cytolysis.

To determine DKK1 as a TAA in MM, we identified and synthesized 2 DKK1 peptides, P20 and P66, after searching DKK1 sequence for HLA-A*0201 binding motifs. To enhance their binding affinity, heteroclitic peptides for P20 (Py20) and P66 (P66v) were synthesized. Because P20 and Py20 had similar binding affinity, and P66v had much higher binding affinity than P66, we used P20 and P66v in our experiments. These peptides were immunogenic in vivo. After a single immunization of HLA-A*0201 transgenic mice with the peptides, splenocytes from immunized mice contained detectable peptide-specific T cells, analyzed as peptide-tetramer-positive and IFN-γ-secreting CD8+ T cells that were able to kill target cells. We detected, by DKK1 peptide-tetramer staining, naturally occurring DKK1-specific CD8+ T cells in the PBMCs of patients with myeloma. We have evidence...
to indicate that DKK1 is a shared TAA among different patients. In this study, we focused on DKK1 peptides presented by HLA-A*0201 molecules. The finding that DKK1 peptide-specific CTLs recognized and lysed autologous and allogeneic DKK1/HLA-A*0201 + myeloma cells, but not DKK1−/HLA-A*0201 + or DKK1−/HLA-A*0201 − myeloma cells, supports this notion. Furthermore, our findings indicate that these DKK1 peptides are also naturally processed and presented by myeloma cells in the context of surface MHC class I molecules.

CTL recognition of target cells via their T-cell receptors activates 2 distinct mechanisms of cell lysis.40,41 The first is granule exocytosis, mediated by the pore-forming perforin and granzyme A and B. The second involves interaction between the FasL on effector cells and Fas molecules expressed on the target cells. In the present study, the CTLs seemed to lyse the target cells, mainly via the perforin-mediated pathway, because the cells expressed high levels of granzyme B and perforin but not FasL. These findings are of special importance in view of published results on Fas expression on myeloma cells. A previous study showed that Fas antigen point mutation was detected in 10% of patients’ bone marrow samples. The mutations were located in the cytoplasmic region that is involved in transduction of an apoptotic signal and, thus, rendered the cells resistant to Fas-induced apoptosis.42 Furthermore, myeloma cells induced to be drug resistant also became resistant to Fas-mediated apoptosis.42 Thus, the use of CTLs that are cytotoxic via the Fas-mediated pathway may be limited, but the pore-forming CTLs can be used for the treatment of drug-resistant myeloma.

In conclusion, our study demonstrates that DKK1 peptide-specific CTLs can be generated by stimulating autologous T cells with DCs pulsed with DKK1 peptides. These CTLs may be promising effector cells for immunotherapy in MM because they are potent killer cells able to specifically and effectively lyse myeloma cells, including primary myeloma cells but not normal blood cells in vitro. Our study indicates that DKK1 peptides may be used as universal vaccines to immunize patients. Thus, this study is the first to provide strong and direct evidence to support the application of DKK1-based immunotherapy in MM.

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Authorship

Contribution: J.Q. and Q.Y. initiated the work, designed the experiments, and wrote the manuscript. J.Q., J.X., S.H., J.Y., L.Z., and X.H. performed the experiments and statistical analyses. M.W., F.Z., J.D.S., J.E., and L.W.K. provided samples and critical suggestion to this study.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Qing Yi, Department of Lymphoma and Myeloma, M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 0903, Houston, TX 77030; e-mail: qyi@mdanderson.org.

References


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