Therapy of Murine Tumors with p53 Wild-type and Mutant Sequence Peptide-based Vaccines

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Summary

The BALB/c Meth A sarcoma carries a p53 missense mutation at codon 234, which occurs in a peptide, termed 234CM, capable of being presented to cytotoxic T lymphocytes (CTL) by H-2K^d molecules (Noguchi, Y., E.C. Richards, Y.-T. Chen, and L.J. Old. 1994. *Proc. Natl. Acad. Sci. USA.* 91:3171–3175). Immunization of BALB/c mice with bone marrow–derived dendritic cells (DC), generated in the presence of granulocyte macrophage colony-stimulating factor and interleukin 4, and prepulsed with the Meth A p53 mutant peptide, induced CTL that specifically recognized peptide-pulsed P815 cells, as well as Meth A cells naturally expressing this epitope. Immunization with this vaccine also protected naive mice from a subsequent tumor challenge, and it inhibited tumor growth in mice bearing day 7 subcutaneous Meth A tumors. We additionally determined that immunization of BALB/c mice with DC pulsed with the p53 peptide containing the wild-type residue at position 234, 234CW, induced peptide-specific CTL that reacted against several methylcholanthrene-induced BALB/c sarcomas, including CMS4 sarcoma, and rejection of CMS4 sarcoma in vaccination and therapy (day 7) protocols. These results support the efficacy of DC-based, p53-derived peptide vaccines for the immunotherapy of cancer. The translational potential of this strategy is enhanced by previous reports showing that DC can readily be generated from human peripheral blood lymphocytes.

During the past few years, a number of CTL-defined, tumor-related peptides have been identified in human cancers, and attempts are being made for their clinical application (1–6). An essential aspect of peptide-based vaccine development is the identification of adjuvants that facilitate the induction of antitumor immune responses, particularly antitumor CTL. These cells have been shown to be crucial effectors in antitumor immune responses in preclinical as well as clinical settings (7). Optimal induction of CTL is believed to require an initial encounter with antigenic peptides presented by so-called "professional" APC. Dendritic cells (DC)[1] are presently considered the most potent APC, based in part on the demonstrated ability of peptide-pulsed DC to prime naive CTL in vitro and in vivo (8–10). As such, DC offer a potentially promising vehicle for the delivery of immunotherapeutic peptides in the treatment of cancer and infectious diseases (11).

Preclinical models are needed, however, to guide the clinical development of DC-based immunotherapy for cancer patients. Immunization with bone marrow (BM)-derived DC (12, 13) pulsed with peptides capable of being recognized by CTL induces a strong specific CTL response in several mouse models tested (14–17). Using CTL-defined peptides derived from OVA and human papilloma virus 16 (HPV16) E7 protein, as well as unfractionated class I MHC–binding peptides acid eluted from the chemically induced C57BL/6 MCA205 sarcoma, we have already demonstrated that administration of peptide-pulsed, DC-based vaccines can immunize mice in vaccine and therapy settings (14–16). The recent description of simple methods to generate large numbers of DC from human PBL cultured in the presence of GM-CSF pls IL-4 and their in vitro use to obtain CTL (18, 19) suggests the feasibility of DC-based vaccine strategies for cancer immunotherapy in humans.

The majority of antitumor cellular responses presently characterized in humans involve CTL directed against self peptides derived from ectopically expressed or lineage-spe-

[1] *Abbreviations used in this paper:* BM, bone marrow; CM, complete medium; CS, circumsporozoite; DC, dendritic cells; LLO, listeriolysin O.

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cific cellular proteins (5, 6, 20). Coulie et al. and Wolfel et al. recently described two distinct melanoma epitopes arising from point mutations, though the appearance of these epitopes thus far are confined to tumors of individual patients (21, 22). Missense mutations in the p53 gene occur in approximately half of human cancers (23), and p53-derived peptides may represent ideal targets for cellular immunotherapy applicable to a wide range of patients (24, 25). While peptides encompassing point mutations offer the potential of serving as tumor-specific epitopes, this potential rests on the occurrence of a mutation within a peptide that can be processed and presented to CTL by a given class I allomorph. Alternatively, it might be reasoned that since mutant p53 gene products are often overexpressed in tumors (23, 26, 27), enhanced presentation of p53-derive peptides from nonmutated portions of the molecule might accompany protein overexpression. Consequently, an optimistic scenario arises in which, by analogy with responses observed to lineage-specific epitopes, tumor-selective cellular responses might be elicited to wild-type p53 peptides, independent of the p53 mutation in an individual of a given MHC haplotype.

We sought to determine whether DC-based vaccines that use both mutant and wild-type, p53-derived peptides could effectively elicit antitumor CTL in mice challenged with established methylcholanthrene-induced sarcomas. Noguchi et al. (28, 29) recently described the use of Meth A sarcoma p53-mutant and wild-type peptide ($p53_{(232-240)}$), designated 234CM and 234CW, respectively, in vaccination protocols using BALB/c mice. Mutant peptide 234CM-based vaccines that use incomplete Freund's adjuvant (IFA) or QS-21 adjuvant and IL-12 were shown to induce antipeptide CTL, while only the QS-21/IL-12 vaccine induced Meth A rejection in vaccination and therapy settings. Here, we assess the efficacy of immunization with syngeneic BM/DC pulsed with the 234CM peptide to induce CTL capable of recognizing Meth A sarcoma presenting the naturally processed epitope, as well as peptide-pulsed target cells, and tumor rejection in vaccination and therapy settings. The results of these studies confirmed the potential of peptide/DC vaccines to induce effective antitumor immune responses, and permitted us to extend our studies to an analysis of the efficacy of the p53 wild-type peptide 234CW/DC vaccine against several chemically induced BALB/c sarcomas known to be overexpressing p53.

Materials and Methods

Mice. BALB/cJ and BALB/c × C57BL/6 (CB6F1) female mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in a specific pathogen-free facility.

Tumors and Cell Lines. The chemically induced BALB/c Meth A ascites sarcoma, CMS series of chemically induced BALB/c sarcomas, and cell lines derived from them have been described previously (30). The DBA/2 mastocytoma P815 was obtained from the American Type Culture Collection (ATCC, Rockville, MD). All the chemically induced sarcomas of BALB/c origin used in this study express mutated p53 (26, 27, 31). The Meth A sarcoma has three p53 mutations, at codons 132, 168, and 234 (31), while CMS1 contains a mutation in codon 239 (Cys to Phe) (27).

Antibodies. Supernatants containing mAb produced by the following hybridomas, which were obtained from ATCC, were used in this study: anti-$H\text{-}2K^d$ (HB159), $-H\text{-}2D^d$ (HB104), -CD4 (TIB207), -CD8 (TIB221), and -B220 (TIB146).

Generation of BM-derived DC. BM cells, which had been immunodepleted of lymphocytes by treatment with anti-B220, -CD4, and -CD8 mAb and rabbit serum as C source, were cultured overnight in 24-well plates (Corning Inc., Corning, NY) to remove adherent cells at a cell density of 10^6 cells/well per ml complete medium (CM: RPMI 1640 supplemented with 10% FCS, antibiotics, glutamine, and 50 μM 2-ME). All reagents were obtained from GIBCO BRL, Gaithersburg, MD). The nonadherent cells were harvested and cultured at a cell density of 2.5×10^5 cells/well per ml CM supplemented with GM-CSF (10^3 U/ml; Sigma Immunochemicals, St. Louis, MO) alone or in combination with either TNF-α (100 U/ml; Genzyme, Boston, MA) or IL-4 (10^3 U/ml; Schering-Plough, Kenilworth, NJ) in 24-well plates. Cells were harvested 8 d later (12, 13).

Peptides. Synthetic p53-derived peptides containing wild-type and mutant residues, as determined by Arai et al. (31) and designated as in Noguchi et al. (28), were used in this study. The wild-type peptide $p53_{(232-240)}$, 234CW, has the sequence KY**M**-CNSSCM, while that of the mutant peptide, 234CM, is KY**I**-CNSSCM. The CMS1 p53 peptide containing the missense mutation at codon 239 as determined by Halevy et al. (27), is KYMCNSS**F**M. In accordance with the nomenclature of Noguchi et al. (28), this peptide is designated 239CM. Other p53-derived peptides used were $p53_{(130-138)}$ (LF**C**QLAKTC, 132CW; LF**F**QLAKTC, 132CM) and $p53_{(166-174)}$ (MT**E**VVRRCP, 168CW; MT**G**VVRRCP, 168CM) (28). The $H\text{-}2K^d$–binding *Plasmodium bergii* circumsporozoite (CS)-derived peptide, DSYIPSAEKI, was also used in these studies (32). Additional peptides used in binding assays, described below, include the listeriolysin O (LLO)-derived peptide $LLO_{(91-99)}$ (G**Y**KDGNEYI), an LLO analogue, LLO(Y2F) (G**F**KDGNEYI), and the OVA-derived peptide $OVA_{(257-264)}$ (SIINFEKL) (33). All peptides were synthesized by standard F-moc chemistry and purified by HPLC.

Radiolabeled Peptide Binding. Peptide $LLO_{(91-99)}$ was modified by replacing Tyr at P2 with Phe to produce the peptide LLO(Y2F), containing a single Tyr at P8 available for iodination by the chloramine T method. $LLO_{(91-99)}$ has been shown previously to bind to $H\text{-}2K^d$ with high affinity (33); in the present assay system, the substituted analogue LLO(Y2F) was found to bind with an affinity comparable to that of the parent peptide. Competitive inhibition of ^{125}I-LLO(Y2F) binding to $H\text{-}2K^d$ molecules on live cells was performed according to a previously published method (34). Specific binding is defined as the cpm bound in the absence of cold peptide minus the cpm bound in the presence of 100 μg/ml cold LLO(Y2F).

Reverse Transcription and PCR (RT-PCR). Total cellular RNA was prepared using the RNeasy kit (Qiagen Inc., Chatsworth, CA). cDNA synthesis was performed using the Superscript Preamplification System from GIBCO BRL (Gaithersburgh, MD) as previously described (35). For each reaction, 2 μg of RNA were reverse transcribed using random hexamer according to the protocol supplied by GIBCO BRL. The cDNA was amplified using 100 pmol each of p53.1 (5′-ATG.TGC.ACG.TAC.TCT.CCT-3′) and antisense p53.2 (5′-GTG.GAT.GGT.GGT.ATA.CTC-3′) for amplification from bp 360 to 692, which represents amplification from the expressed product of the 3′ end of exon 4 to the 5′ end of exon 7 (the sequence of primer p53.1 is from exons 4 and

5) and p53.3 (5′-GAG.TAT.ACC.ACC.ATC.CAC.-3′) and antisense p53.4 (5′-TTC.TTC.TTC.TGT.ACG.GCG-3′) for amplification from bp 675 to 855, which represents amplification from the expressed product of the 5′ end of exon 7 to the middle of exon 8 (31, 36). Samples were also reverse transcribed and amplified using primers specific for actin (Clontech Laboratories, Palo Alto, CA) to verify that the RNA was intact and could be reverse transcribed and amplified. Samples were amplified for 30 cycles in a DNA Thermal Cycler (Perkin-Elmer, Norwalk, CT) using the following conditions: 94° for 1 min, 49° for 2 min, 72° for 3 min, followed by 15 min at 72°. PCR products were identified on ethidium bromide–stained 1% agarose gels.

Cloning and DNA Sequencing. cDNA products were ligated directly into the pCRII vector using the TA cloning kit (Invitrogen, San Diego, CA) or ligated after isolation of specific bands from 1% low melting point agarose (FMC BioProducts, Rockland, ME) gels. Plasmid DNA was prepared after liquid culture of isolated recombinant colonies using standard mini-prep methods. Double-stranded DNA was sequenced on both strands by the dideoxy chain termination methods using Sequenase™ Version 2.0 (U.S. Biochemical Corp., Cleveland, OH) with the M13 reverse-sequencing primer and T7 sequencing primer.

Immunization of Mice with DC-based Peptide Vaccines. BM-derived DC, grown in the presence of GM-CSF alone or in combination with either TNF-α or IL-4, were incubated with peptides at a concentration of 10 μg/ml per 10^6 DC/ml CM for 2 h at 37°C. The cells were harvested by centrifugation and washed with PBS, and irradiated (3,000 rad) before use. Groups of three to five mice received two weekly intravenous injections of 10^5 DC or peptide-pulsed DC (14). 7 d after the last immunization, mice were killed and their spleens removed for in vitro restimulation or challenged bilaterally subcutaneously with in vivo–grown Meth A sarcoma, 3.5×10^5 cells, or CMS4, 1.5 or 2.5×10^5 cells. In the Meth A tumor model, the efficacy of DC-based peptide vaccines was directly compared to that of IFA admixed with the 234CM peptide. Tumor growth was monitored every 7 d. Fisher's exact method or the Student's *t* test were performed to interpret the differences between experimental groups (presented as mean ± SEM). Two-sided *P* values (*t* test) or significance at 95% confidence limits are presented for individual experiments.

In Vitro Restimulation with Peptides. Groups of mice that had been immunized with various peptide-based vaccines were killed 1 wk after the last immunization, and their spleens were removed for in vitro restimulation by peptide-pulsed splenocytes prepared by the addition of 10 μg peptide to 10^7 splenocytes in 1 ml CM followed by a 3-h incubation at 37°C. The nonadherent lymphocytes obtained from each experimental group were restimulated at a cell density of 10^6 lymphocytes/4×10^6 peptide-pulsed splenocytes per 2 ml CM in 24-well plates. The cultures were incubated in a 5% $C0_2$–humidified atmosphere for 7 d and harvested.

Cell-mediated Cytotoxicity Assays. In vitro–restimulated lymphocytes were tested for their cytolytic reactivity against BALB/c sarcomas, P815 and peptide-pulsed P815 target cells in standard 4-h ^{51}Cr release assays using 96-well round-bottomed plates. Target cells were radiolabeled with 100 μCi Na_2-$^{51}CrO_4/2 \times 10^6$ cells for 1 h at 37°C; peptide-pulsed P815 cells were prepared by incubating P815 cells with the radiolabel and peptide at a concentration of 10 μg/ml. In mAb-blocking experiments, hybridoma supernatants were added at a final dilution of 1:10. Maximum and spontaneous release, as well as the percentage of specific ^{51}Cr cpm release, were determined by standard procedures.

Treatment of Tumor-bearing Mice with Peptide-pulsed Vaccines. Groups of five mice each bearing established 7-d-old sarcomas were injected intravenously with 10^5 DC(GM-CSF/IL-4) either unpulsed or pulsed with 10 μg/ml peptide 7- and 14-d after challenge. Tumor growth was monitored as detailed above.

Results

Binding of p53-drived Peptides to Class I MHC. Peptide-binding inhibition experiments confirmed that 234CM, 234CW, and 239CM p53 peptides are capable of binding to H-2K^d (Fig. 1). Inhibition was observed for both the 234CW and 234CM peptides, in addition to the positive controls LLO/LLO(Y2F). No inhibition was observed for the p53-derived peptides 132CW/CM and 168CW/CM, consistent with their lack of immunogenicity, as previously reported (28). The data indicated that 234CM is a poorer binder than 234CW or 239CM peptides, producing only 30% inhibition of ^{125}I-LLO(Y2F) binding at the relatively high concentration of 100 nM. Based on reported studies examining binding of various peptides to a number of class I allomorphs (37–39), peptides exhibiting high affinity binding normally produce half-maximal inhibition at a concentration (IC_{50} value) well below 100 nM, while peptides binding with moderate to low affinity have IC_{50} values near or above 100 nM.

Tumor Rejection–inducing Activity of Peptide-pulsed DC-based Vaccines. BM-derived DC generated in the presence of GM-CSF alone or in combination with TNF-α or IL-4 were pulsed with the 234CM and 234CW peptides and evaluated for their efficacy in protecting mice from a lethal

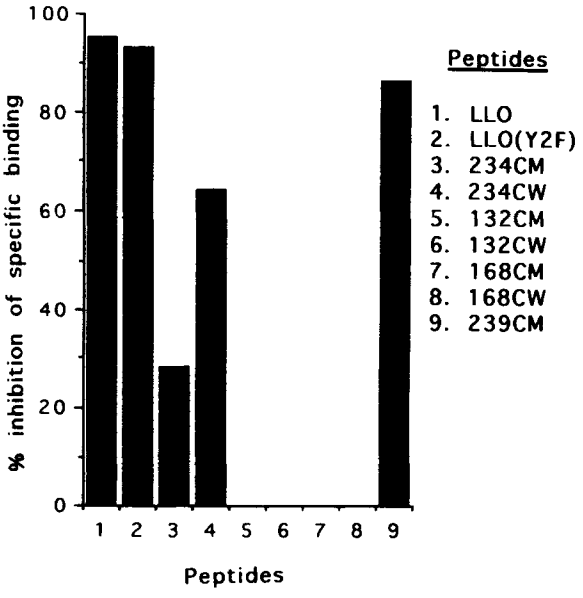

Figure 1. Competitive inhibition of ^{125}I-LLO(Y2F) binding to H-2K^d molecules on P815 cells. Peptides at a concentration of 100 nM were tested for their capacity to inhibit binding of a trace concentration (<0.5 nM) of radiolabeled peptide. The LLO/LLO(Y2F) and the H-2K^b–presented peptide OVA were used as the postive and negative controls for this assay. Total and nonspecific cpm bound for the experiment shown were 9,947 and 88 cpm, respectively.

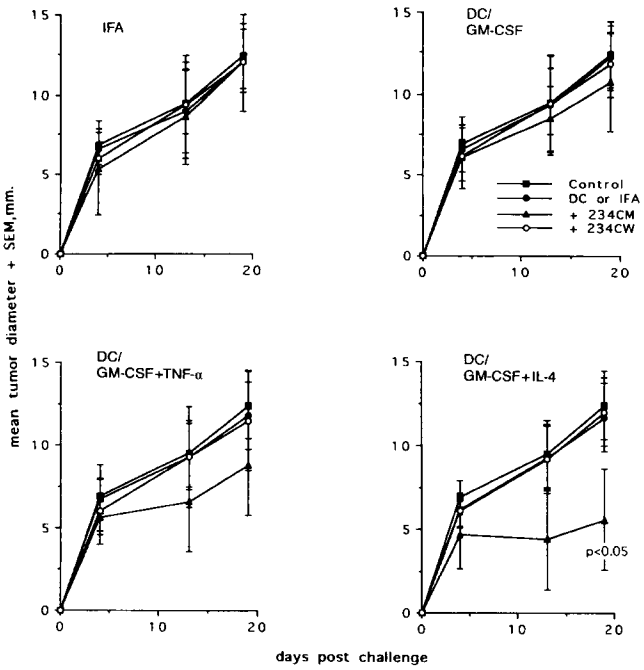

Figure 2. Meth A p53 234CM peptide–DC vaccine protects BALB/c mice from a subsequent challenge with Meth A sarcoma. Groups of five mice each were immunized twice by intravenous injection with vaccines consisting of the 10 μg/ml 234CM peptide admixed with IFA or pulsed onto 10^5 BM-derived DC generated in the presence of GM-CSF, GM-CSF/TNF-α, or GM-CSF/IL-4, before challenge with 3.5×10^5 Meth A sarcoma. Tumors were measured on the indicated days. Data are representative of three experiments performed.

challenge of the Meth A sarcoma (Fig. 2). All three populations of BM-derived DC used in this experiment express elevated levels of class II MHC and costimulatory molecules, B7.1 and B7.2 (13). The DC(GM-CSF/IL-4), however, express higher levels of these antigens than the other two DC populations. In addition, DC(GM-CSF/IL-4) are more potent stimulators of the allogeneic MLR than either DC generated in the presence of GM-CSF alone or in combination with TNF-α (14, 15). Similarly, human DC generated in the presence of GM-CSF and IL-4 have been shown to be more effective stimulators of an MLR than DC(GM-CSF) or (GM-CSF/TNF-α) (19). The dose of peptide-pulsed DC used in this experiment was 10^5 cells/immunization. It was chosen primarily on basis of the previously reported ability of this number of peptide-pulsed DC to induce antipeptide CTL (10, 17). A higher dose of peptide-pulsed DC (10^6) had been shown to induce nonspecific cytotoxic effector cells (17). Controls for these experiments included untreated mice, as well as those immunized with IFA alone or admixed with either the 234CM or 234CW peptide.

Of the 12 types of vaccines tested, the only one found to be effective in protecting mice against Meth A tumor challenge consisted of DC(GM-CSF/IL-4) pulsed with the 234CM peptide (Fig. 2). Although 234CM/DC(GM-CSF/TNF-α) enhanced the resistance of the mice to tumor challenge, its effect was not statistically significant. Mice immunized with vaccines consisting of 234CM pulsed onto GM-CSF–generated DC or admixed with IFA were ineffective, as were all of the wild-type 234CW-pulsed DC vaccines.

Induction of Anti-Meth A CTL by Peptide-pulsed DC Vaccines. Based on the proven efficacy of the 234CM-pulsed DC (GM-CSF/IL-4) vaccine in protecting mice against Meth A sarcoma, we analyzed similarly immunized mice for induction of anti–Meth A CTL. Controls for this analysis included lymphocytes obtained from naive mice and mice treated with DC alone or 234CM admixed with IFA. Splenocytes obtained from these groups of mice were restimulated in vitro with 234CM-pulsed splenocytes, and the resulting effector cells were evaluated for their cytolytic reactivity against P815, 234CM-pulsed P815, and Meth A target cells. Antipeptide CTL were obtained from mice immunized with either 234CM-DC or 234CM-IFA vaccines (Fig. 3). Only

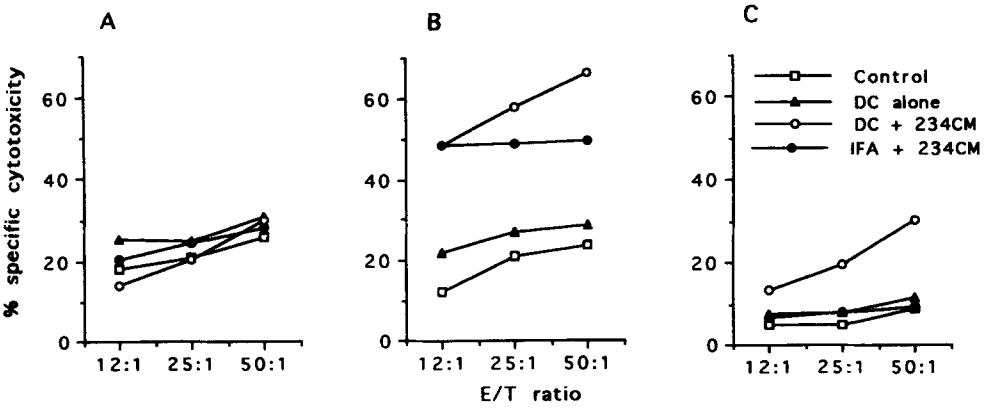

Figure 3. Cytotoxic reactivities of effector cells induced in mice by immunization with Meth A p53 mutant peptide–based vaccines. The vaccines used consisted of the 234CM peptide admixed with IFA or pulsed onto DC(GM-CSF/IL-4). Splenocytes (A–C) obtained from the various groups of immunized mice were restimulated in vitro with 234CM-pulsed splenocytes. The cytotoxic reactivity of effectors against P815 (*A*), 234CM-pulsed P815 (*B*), and Meth A (*C*) targets was determined in standard 4-h ^{51}Cr release assays at the E/T ratios indicated. Data are representative of three experiments performed. The SEM was in all cases <15% of the mean values.

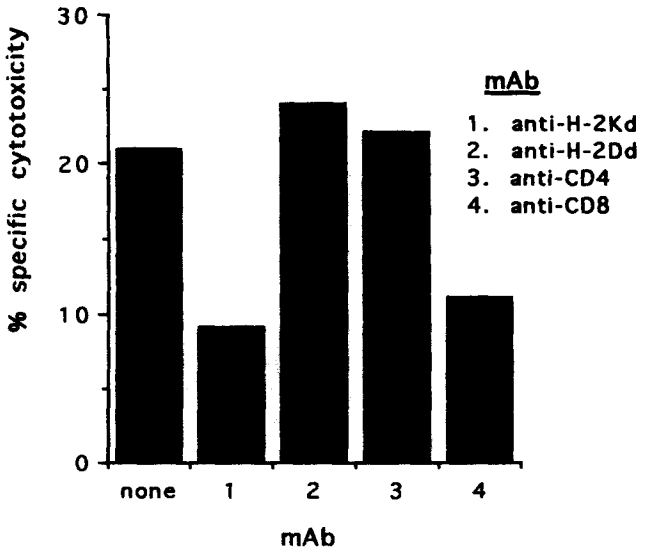

Figure 4. Blocking of the cytotoxic activity of anti–Meth A CTL by mAb. The anti–Meth A CTL were tested at an E/T ratio of 50:1, and in the presence of hybridoma supernatants at a final dilution of 1:10.

the 234CM-DC vaccine, however, induced effectors with cytolytic reactivity against Meth A target cells (Fig. 3). The anti–Meth A reactivity of these effectors was blocked by anti–H-2K^d and -CD8 mAb, but not anti–H-2D^d or -CD4 mAb (Fig. 4).

Meth A expresses two p53 transcripts: one encodes the mutations at codons 168 and 234, while the other encodes the mutation at codon 132 (30). Consequently, the Meth A sarcoma has the potential to express epitopes containing either the wild-type or mutant 234 codon. The inability of the 234CW-based vaccines to induce an anti–Meth A immune response suggests, however, that either the 234CW/DC vaccine did not induce antipeptide CTL or the level of this epitope presented by Meth A sarcoma is not sufficient for effective recognition by anti-234CW CTL.

Immunotherapeutic Effects of Administration of 234CM-pulsed DC vaccines on Meth A–bearing Mice. The effect of the 234CM-pulsed DC vaccine on the growth of established Meth A tumors was evaluated after it was administered to mice 7 and 14 d after Meth A challenge. As shown in Fig. 5, treatment of five Meth A–bearing mice with this vaccine induced tumor rejection in three of the mice and inhibition of tumor growth in the remaining two mice. Administration of DC alone or DC pulsed with the 234CW peptide had no beneficial effect on Meth A–bearing mice.

Induction of Antitumor CTL by 234CW-pulsed DC Vaccine. We questioned whether anti-234CW CTL could be induced, and if so, whether they would recognize tumors overexpressing p53. We determined that immunization of mice with a 234CW/DC vaccine did induce effectors capable of recognizing 234CW-pulsed P815 target cells. Interestingly, they also recognized untreated P815 cells. They did not recognize Meth A sarcoma or 234CM-pulsed P815 cells. Furthermore, recognition of 234CW-pulsed P815 targets was inhibited by the H-2K^d–binding CS peptide, but not by the nonbinding p53 168CM peptide (Fig. 6 *A*). These results support the specificity of these effectors for the wild-type or "self" p53 epitope, and they indicate that P815 cells naturally process this epitope. The anti-234CW effectors were also tested against a panel of non–cross-reacting, chemically induced BALB/c sarcomas that overexpress p53 (26, 27, 30). The panel consisted of CMS1, CMS3, CMS4, and CMS5, and the effectors recognized all of the sarcomas except CMS1 (Fig. 6 *B*). Recognition of the three sarcomas was blocked by anti–H-2K^d mAb, but not anti–H-2D^d mAb, indicating that the effectors were class I MHC restricted. The tumor not recognized by these CTL, CMS1, expresses a mutation at codon 239 (27). In a separate experiment, we determined that the 239CM peptide induced CTL reactive against CMS1 but not Meth A (data not shown).

One presumes that the four tumors recognized by the anti-234CW CTL express elevated levels of p53 molecules with missense mutations occurring at codons other than 232–240. This would permit the cells to present the 234CW epitope at a level sufficient for it to be recognized by the CTL. This hypothesis was confirmed for CMS4 sarcoma. The RT-PCR product of CMS4 p53 exons 5 and 6 was

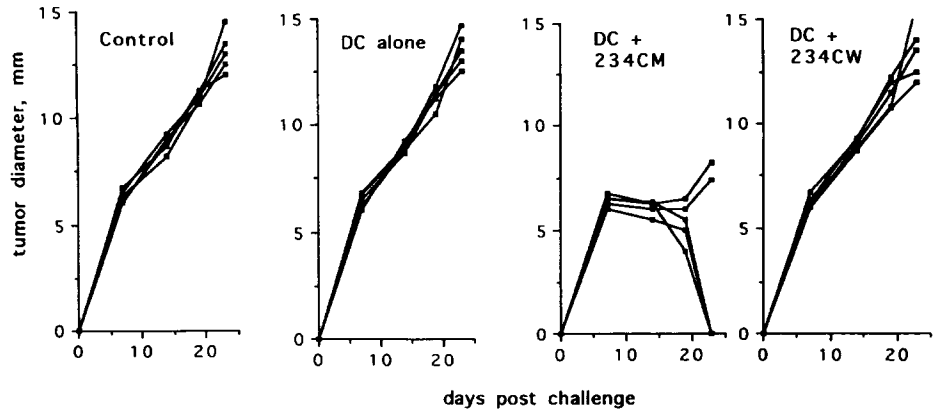

Figure 5. The effect of administration of 234CM/DC vaccine on mice bearing 7-d established Meth A sarcoma. Groups of five BALB/c mice were challenged with 3.5 × 10^5 Meth A. The mice were treated 7 and 14 d later by intravenous injection of 10^5 irradiated DC(GM-CSF/IL-4) alone or pulsed with 10 μg/ml 234CW or 234CM peptide. Untreated tumor-bearing mice were controls for this experiment. Data are representative of two experiments performed.

found to contain a missense mutation at codon 194 (G to C; Val to Ala), while the RT-PCR products of exons 7 to mid-8 were wild type.

Since thymocytes, as well as mitogen-stimulated lymphocytes, have been shown to express increased levels of p53 (40), we asked whether (*a*) Con A–stimulated splenocytes would be recognized by 234CW-induced CTL; and (*b*) immunization of mice with 234CW/DC vaccines would have an immunosuppressive effect on the mice. We determined that Con A–stimulated splenocytes were not recognized by anti-234CW CTL (8.3 ± 1.1% vs 19.1 ± 2.8% for CMS4 and 3.6 ± 0.5% for Meth A cells at an E/T ratio of 50:1) and that 234CW/DC–immunized mice maintained for 4 mo remained visibly healthy throughout this time period. Moreover, immunization of 234CW-immune mice with the unrelated H-2K^d–binding CS peptide induced CTL reactive against CS-pulsed P815 cells at a level comparable to that induced in control mice (49.2 ± 6.7% for 234CW-immune mice vs 52.7 ± 7.4% for CS-immunized control mice at an E/T ratio of 50:1).

Tumor Rejection-inducing Activity of 234CW-pulsed DC-based Vaccines. Based on the observed cytotoxic reactivity of anti-234CW CTL for CMS4 sarcoma, DC(GM-CSF/IL-4) pulsed with the 234CW peptide were evaluated for their efficacy in protecting mice from a lethal challenge with the CMS4 sarcoma. Controls for these experiments included untreated mice, as well as mice immunized with DC alone or 234CM-pulsed DC. The only vaccine found to be effective in protecting mice against the CMS4 tumor challenge consisted of DC pulsed with the 234CW peptide (Fig. 7).

Immunotherapeutic Effects of Administration of 234CW-pulsed DC Vaccine to CMS4-bearing Mice. The effect of the 234CW-pulsed DC vaccine on the growth of established CMS4 tumors was evaluated after its administration to mice 7 and 14 d after CMS4 challenge. As shown in Fig. 8, treatment of five CMS4-bearing mice induced tumor rejection in three of the mice and inhibition of tumor growth in the remaining mice. Administration of DC alone or DC pulsed with either the 234CM or CS peptide had no beneficial effect on CMS4-bearing mice.

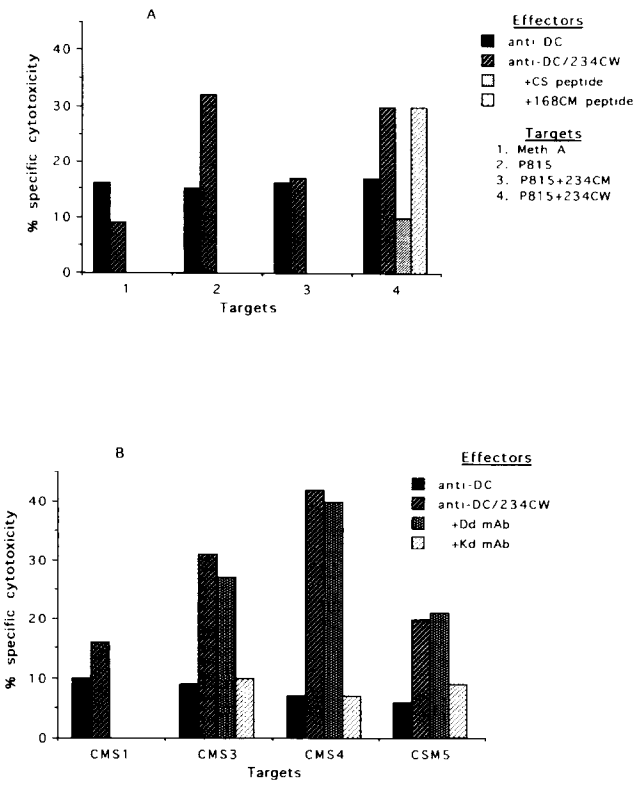

Figure 6. Analysis of cytolytic activity of effectors induced in BALB/c mice by p53 234CW/DC vaccine. (*A*) Peptide inhibition of cytotoxic reactivity against P815 and peptide-pulsed P815 targets. (*B*) Cytotoxic reactivity against chemically induced BALB/c sarcomas and blocking of cytotoxic reactivity by mAb. All assays were done at an E/T ratio of 50:1. Data are representative of three experiments performed. The SEM was in all cases <15% of the mean values.

Discussion

We have demonstrated that administration of vaccines consisting of H-2K^d–binding wild-type or mutant p53$_{(232\text{-}240)}$ peptides pulsed onto BM-derived DC were highly effective in inducing antitumor CTL and tumor resistance in

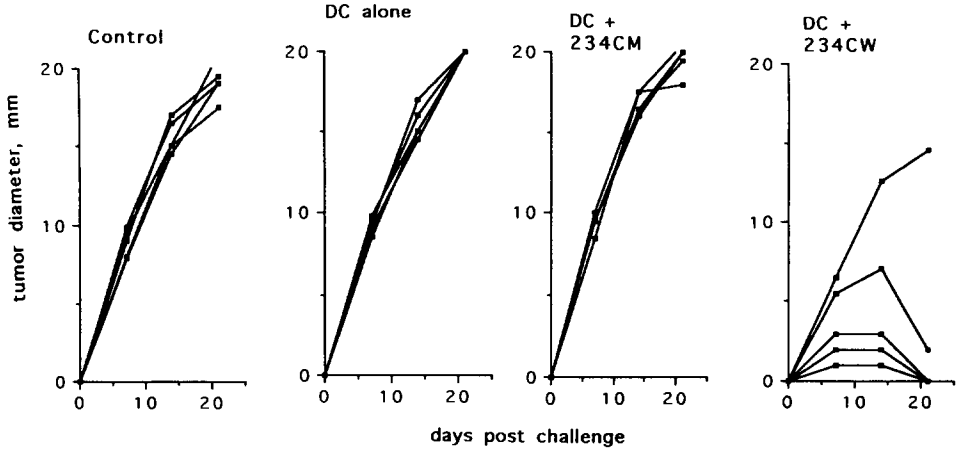

Figure 7. p53 wild-type 234CW peptide–DC vaccine protects BALB/c mice from a subsequent challenge with CMS4 sarcoma. Groups of five mice each were immunized twice by intravenous injection with vaccines consisting of 10^5 DC(GM-CSF/IL-4) alone or pulsed with 10 μg/ml mutant 234CM or wild-type 234CW peptide before challenge with 2.5 × 10^5 CMS4 sarcoma. Data are representative of two experiments performed.

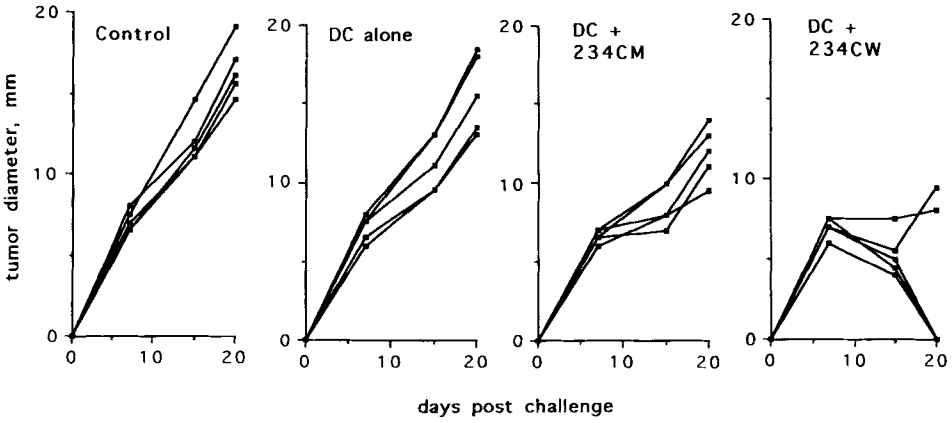

Figure 8. The effect of administration of wild type p53 peptide vaccine on mice bearing 7-d established CMS4 sarcoma. Groups of five BALB/c mice were challenged with 1.5×10^5 CMS4. The mice were treated 7 and 14 d later by intravenous injection of 10^5 irradiated DC(GM-CSF/IL-4) alone or pulsed with 10 μg/ml 234CW or 234CM peptide. Untreated tumor-bearing mice were controls for this experiment. Data are representative of two experiments performed. The mean tumor diameter of mice treated with the 234CW/DC vaccine was significantly smaller than that of the control groups ($P < 0.05$).

mice. Furthermore, treatment of tumor-bearing mice with these vaccines was shown to have beneficial therapeutic effects. Previously, Noguchi et al. reported the successful induction of p53 mutation–specific CTL using the mutant Meth A p53 peptide. The vaccines used consisted of the 234CM peptide admixed with IFA or QS-21 (28, 29). As observed in similar attempts to induce in vitro or in vivo antitumor CTL, the resulting CTL were capable of recognizing peptide-pulsed target cells, but apparently not target cells expressing the naturally processed epitope (41).

Dendritic cells are known to be highly effective APC (8). In the present study, we directly compared the efficacy of peptide-based vaccines consisting of three types of BM-derived DC populations (those generated in the presence of GM-CSF alone or in combination with TNF-α or IL-4), with IFA admixed with the Meth A p53 mutant 234CM peptide. As with other models we have analyzed, vaccines using DC(GM-CSF/IL-4) were the most effective in inducing antitumor immune responses (14). This finding underscores the importance of effective adjuvant/delivery systems for the generation of CTL of sufficient affinity to recognize the naturally processed epitope, and DC appear to be the vehicle of choice in this regard. It is worth noting that the phenotype and function of the murine DC used in this study are comparable to those of human blood–derived DC grown in the presence of GM-CSF and IL-4 (19).

Because of the high frequency of mutations occurring in the *p53* gene in human tumors, the product of this mutated gene is an attractive candidate for tumor peptide–based vaccines and immunotherapy. The applicability of anti-p53 based immunotherapy of cancer might be greatly enhanced, however, if p53 wild-type or "self" epitopes, as well as mutant "nonself" epitopes, could be used as immunogens. Our success in using a class I MHC–binding p53 wild-type epitope to induce tumor resistance suggests that p53 wild-type epitopes might be used in peptide-based immunotherapy of human cancer. The translational potential of such therapy is enhanced by the fact that HLA-A2.1–binding human p53 wild-type and mutant epitopes have been identified (24, 25), although it is presently not known whether they are naturally processed and presented. Furthermore, while we have observed that induction of anti-p53 wild-type CTL responses had no obvious deleterious effects in naive mice, the potential for inducing an inappropriate response directed to nontransformed cells in vivo warrants further study.

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