Strategies for Antigen Loading of Dendritic Cells to Enhance the Antitumor Immune Response

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ABSTRACT

Dendritic cells (DCs) primed with tumor antigens can effectively mediate the regression of a variety of established solid malignancies in both murine and human models. Despite such clinical efficacy, the optimal means of DC priming is unknown. The goal of this study was to compare three methods of tumor preparation: irradiation, boiling, or freeze thaw lysis for DC priming. Mouse bone marrow-derived DCs were loaded with defined ratios of E.G7 tumor cells expressing a model tumor antigen, OVA. Sensitized DCs were used for stimulation of OVA-specific CTLs derived from OT-1 T-cell receptor transgenic mice. IFN-γ release, determined by ELISA at 24 and 48 h, was used to assess the expression of antigens by DCs. DCs loaded with irradiated tumors were effective stimulators for OT-1 CTLs, whereas DCs stimulated with freeze-thawed or boiled tumors did not stimulate IFN-γ production. Freeze-thaw lysis appeared to inhibit CTL activity in vitro and in two of three cases, this effect was not overcome by the addition of OVA. The ability to load irradiated tumor cells was reproduced in two analogous human melanoma models using melanoma cell lines expressing gp100 and CTL clones specific for a gp100 melanoma antigen. Consistent with the in vitro data, only DC/irradiated tumor vaccines were effective in preventing or delaying outgrowth of E.G7 and a poorly immunogenic murine squamous cell carcinoma (SCCVII), on local tumor challenge. These data demonstrate that the method of tumor cell preparation clearly influences the ability of DCs to present antigen to T cells. Correlation of in vitro data with the generation of protective immunity in vivo suggests the utility of irradiated tumor-primered DCs as a means to generate protective immunity in patients with solid malignancies.

INTRODUCTION

DCs are professional APCs that direct the cellular immune response through antigen presentation in the presence of appropriate costimulation (1). Recent evidence indicates that DCs primed with tumor antigens can stimulate the regression of established renal cell carcinoma and melanoma (2, 3). Despite such clinical efficacy, a significant percentage of patients with metastatic solid tumors remain unresponsive to immunomodulatory therapy, mandating improved understanding of DC function and antigen priming as a means to enhance clinical efficacy. Multiple techniques of antigen priming have been advocated to create a tumor-specific DC vaccine. Methods of antigen preparation include the creation of tumor lysates, apoptotic bodies, peptides, tumor RNA, tumor derived exosomes, and tumor-DC fusion (2–9). These priming strategies have been guided by the desire to present a wide array of putative tumor antigens in the context of appropriate costimulation. Practical limitations to these strategies include a limited supply of autologous tumor, the need to quantify antigen delivery, and concerns regarding the potential induction of autoimmunity. From a clinical perspective, it remains uncertain which of these techniques is both efficacious in stimulating an antitumor immune response and easily incorporated into clinical practice. The efficacy of whole tumor-based antigen priming of DCs is likely related to both the mechanisms required for antigen presentation by DCs and the cause of tumor cell death. There are several potential mechanisms for DCs to prime the immune response to tumor antigens including cross priming, bystander effects, and DC/tumor cell fusion (10). In the cross-priming model, tumor antigens are processed by DCs and presented in the context of MHCI molecules of DC origin (11). This model is likely of primary import when the tumor is used for DC priming. In the fusion model, tumor cells are fused with DCs so that the tumor cell supplies antigen in the context of appropriate MHCI molecules, and DCs provide the requisite costimulatory molecules to induce T-cell activation (12–17). Although spontaneous tumor cell DC fusion has been demonstrated to occur in vitro, the efficiency is <1%, and spontaneous fusion in vivo has not been well demonstrated. Bystander priming is analogous to fusion, relying on the proximity of tumor cells with DCs to provide the appropriate antigen stimulation and costimulation (10).

Both the mechanism of antigen presentation by DCs and the ability to stimulate antitumor immune T cells are likely influenced by the manner in which tumor cells are processed. Specifically, tumor cells can die by one of two distinct mechanisms, apoptosis or necrosis. Apoptotic cell death is an active process, which occurs as a natural means for the elimination of senescent cells from the body. In contrast, necrosis is directed/mediated by external stimuli such as infection or organ failure (18). Because apoptosis is a natural means of cellular remodeling, several authors have postulated that cells dying in this fashion might not elicit significant cellular immune responses in vivo. In contrast, because necrosis does not occur in the absence of external stimuli, cells dying of necrosis may prove more effective in stimulating a cellular immune response. Importantly, the terms apoptosis and necrosis do not apply to cell death induced by freeze-thaw lysis, which occurs in the absence of danger signals (19).

The goal of this study was to compare three methods of tumor preparation for DC priming: irradiation, boiling, or freeze-thaw lysis. Our results demonstrate that irradiated tumor is most effective in priming DCs for the generation of local protective immunity in a poorly immunogenic murine model for squamous cell carcinoma.

MATERIALS AND METHODS

Animals and Cell Lines. C57BL/6 and C3H mice were purchased from the National Cancer Institute (Frederick, MD). Mice were housed in a specific pathogen-free environment and treated in accordance with the guidelines established by the Animal Care and Use Committee of the Mayo Clinic. The E.G7 thymoma, SCCVII, and B16-OVA cell lines were maintained in CM containing RPMI 1640, 10% heat inactivated fetal bovine serum (Hyclone

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3 The abbreviations used are: DC, dendritic cell; APC, antigen presenting cell; CM, complete medium; GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; FACS, fluorescence-activated cell sorter; PI, propidium iodide; PBMC, peripheral blood mononuclear cell; XRT, X-ray therapy.

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Laboratories, Inc., Logan, UT), penicillin and streptomycin (100 μg/ml), glutamine (2 mM), and HEPES (10 mM).

DCs. Murine DCs were prepared from bone marrow as described previously (20). Mice were sacrificed and the long bone marrow extracted. Cells were suspended in 1 ml of medium containing 10 μg/ml of anti-MHC class II (I-A^b), anti-Mac 3, anti-CD8α (HO2.2), B220, anti-CD3ε, and anti-Gr-1 (all from Pharmingen, Inc.), and incubated on ice for 20 min. The cells were then washed once and resuspended in baby rabbit complement (Accurate Chemical Co., Westbury, NY) at a concentration of 10^7 cells/ml (1:30, complement: medium) at 37°C for 45 min. After incubation in complement, cells were washed and plated in 100-mm cell culture dishes (Falcon, Inc.) at a concentration of 10^7 cells in 10 ml of RPMI 1640 medium supplemented with 10 ng/ml GM-CSF and 1 ng/ml IL-4. Cultures were washed at 2 days to remove nonadherent cells and harvested on day 5.

Human DCs were prepared from the leukaphoresis of an HLA-A2-positive patient after appropriate approval by the Institutional Review Board of the Mayo Foundation. Cells were pelleted and washed in X-Vivo 15 followed by incubation in 175 cm^2 flasks at 1 × 10^7 cells/ml in 25 ml of X-Vivo 15 for 2 h at 37°C in a 5% CO2 atmosphere. Nonadherent cells were removed, and the medium was replaced with 25 ml of X-Vivo 15 containing 500 μl/ml of GM-CSF and 1450 IU/ml of IL-4. After a 6-day incubation, nonadherent cells were removed and adherent cells were then exposed to EDTA. Both nonadherent and adherent cells were washed and combined for FACS analysis and tumor priming. In some instances, DCs were prepared from buffy coat blood from an HLA-A2-positive patient using similar adherence-based enrichment followed by culture in CM-containing GM-CSF and IL-4.

FACS Analysis. The method for FACS analysis has been reported previously (21). Briefly, 10^7 DCs were washed in FACS buffer (10% fetal bovine serum and 0.2% Na Azide in PBS) and stained with conjugated antibodies (PharMingen) for the following markers: HLA-Ⅱ, NLDC 145, CD80, CD86, CD11c, CD40, and Mac 1. After staining, the cells were washed in FACS buffer and fixed (1.0% paraformaldehyde in 0.15 M NaCl). FACS analysis was performed on a FACSscan Flow Cytometer (Becton Dickinson, Inc.). FACS analysis of tumor cells was performed with conjugated PI and Annexin V in accordance with the manufacturer's instructions (PharMingen, San Diego, CA).

DC Priming. DCs were replated on 24-well cell culture plates at a concentration of 10^7 cells/ml in 1 ml of medium. The plate was incubated at 37°C in a 5% CO2 atmosphere during the time-period that tumor cells were being prepared. Either SCCVII or E.G7 cells were prepared at the concentration of 3 × 10^6 cells/well in 0.25 ml of medium. The cells were divided into the following groups: no tumor (DCs alone), irradiated (XRT), boiled (B), and freeze-thawed tumor (FT).

Irradiated tumor received 10 K Rads, whereas boiled tumors were prepared by 10-min incubation at 100°C. A freeze-thawed tumor was processed by freezing the cells in liquid N2 immediately followed by thawing in a 100°C heating block. This procedure was repeated three times in rapid succession. Cells were visually monitored during thawing and were removed from the 100°C block as soon as the suspension entered the liquid phase. Prepared tumor cells were pipetted onto the DCs in the 24-well plate and incubated overnight at 37°C in a 5% CO2 atmosphere. OVA peptide was added at 1 ng/ml to select groups as a positive control. Cells were harvested after 24 h. In vivo experiments were always performed with a tumor:DC ratio of 3:1, whereas this ratio varied for in vitro analysis as described. Additionally, 1 × 10^7 DCs were used for in vitro assays. Importantly, the tumor was aliquoted before preparation to insure that each group received equal amounts.

CTL Assays. In the murine model, inguinal, axillary, and mesenteric lymph nodes were harvested from freshly sacrificed OT-1 transgenic mice. The CTLs were released by gentle smearing between two frosted glass slides. These cells were strained through wire mesh, centrifuged, and separated on nylon wool columns. In select experiments, unfractonated OT-1 LN cells were stimulated in vitro with OVA peptide. In the human model, 624 MEL and MEL888 cells were prepared and used for DC priming in a fashion analogous to that described for the murine tumors. Two human CD8^+ CTL clones specific for an HLA-A2-restricted gp100 epitope were used for these studies. One clone was cultured with 2.5 × 10^3 irradiated (4-5,000 rads) PBMCs, 5 × 10^3 irradiated (15,000 rads) LCL cells, and OKT3 (30 ng/ml) in a T-25 flask containing 25 ml of CM (22). On day 5, 12.5 ml of medium were replaced with 15 ml of fresh medium containing 50 IU/ml of IL-2. CTLs were used for assays on days 9–14. In both the murine and human models, CTLs were added at a 10:1 CTL:DC ratio in 24-well plates to DCs pulsed with tumor manipulated by the methods described above. Supernatants were collected at 24 and/or 48 h, and IFN-γ release was measured by ELISA using curve fit software to determine CTL reactivity. In some instances, the blank control was adjusted to 0 to facilitate reader interpretation.

In Vivo Vaccination. Mice received intradermal injections of 1 × 10^6 DCs loaded with 3 × 10^7 treated tumor cells in a volume of 0.05 ml at weekly intervals × 2. One week after final vaccination, the mice were challenged with local intradermal injections of 1 × 10^6 SCCVII tumor cells in 0.05 ml. Tumors were then measured with metric calipers (Fowler&NSK; Max-Cal) in a blinded fashion at 3–4 days intervals. Palpable tumors were defined as 2 mm (length) by 2 mm (width).

For therapeutic experiments involving the treatment of established tumors, animals were injected i.v. with 2.5 × 10^5 or SCCVII cells suspended in 1 ml of HBSS. Either 4 or 5 days after injection animals were treated with 1 × 10^6 DCs primed with 3 × 10^6 irradiated tumor cells in a volume of 0.05 ml at weekly intervals × 2. In one experiment DCs were matured for an additional 24 h in 5 μg/ml of anti-CD40 before vaccination. Animals were monitored at 1–2 day intervals and sacrificed according to established Institutional Animal Care and Use Committee protocols. Lung metastases were evaluated by necropsy to determine differences in the cause of death between treatment groups.

Statistical Analysis. For statistical analysis of tumor growth, the results of four separate experiments performed in an identical fashion were combined. Not all of the treatment groups were included in each experiment. P[s for comparing the number of mice with tumor growth after tumor challenge were calculated using a two-sided Fisher's exact test.

RESULTS

DC Phenotype. Murine DCs were prepared from bone marrow precursors and harvested for use after 5-day culture in CM containing GM-CSF and IL-4. Human DC precursors were isolated from the PBMCs of an HLA-A2-positive donor after a standard 3-h leukophoresis. The leukophoresis product was additionally separated by brief plastic adherence and cultured for 6 days in GM-CSF and IL-4 before use. In select human experiments, DCs were stored in liquid nitrogen and used after 1-day culture in CM. Before tumor priming, DCs were stained by flow cytometry to analyze surface expression of MHC/HLA molecules, costimulatory molecules, and antigens associated with maturation. Murine DCs expressed high levels of the DC-associated markers NLDC 145 and CD11c, the costimulatory molecules CD80, CD86, and CD40, and MHC class II (Fig. 1A, and data not shown). Human DCs also demonstrated high levels of expression of a nearly analogous panel of markers (Fig. 1B). After culture with irradiated tumor, the tumor vaccine was matured for an additional 24 h with anti-CD40. There was no significant change in either the CD80 or MHC class II expression between DCs cultured with irradiated tumor in the presence or absence of anti-CD40 (data not shown). Preliminary studies also suggest that DCs cultured in the presence of irradiated tumors or lysates have decreased surface expression of B7-1, B7-2, and MHC molecules. These data demonstrate the ability to mature DCs from diverse sources in both animal and human models using plastic adherence followed by brief culture in medium containing IL-4 and GM-CSF. We are currently performing studies to correlate the association of phenotypic changes on DCs with function.

Tumor Antigen Presentation by Murine DCs. An in vitro system was developed to evaluate the ability of DCs to present defined tumor antigens. The OVA expressing E.G7 tumor and B16-OVA were prepared by our methods and pulsed onto DCs at defined ratios. After 24-h incubation, nylon wool purified CTLs from OVA-reactive T-cell receptor transgenic OT-1 mice were added to the cultures. In some experiments OT-1 LN cells were stimulated with OVA before use. Supernatants were collected at 24 and 48 h and assayed for IFN-γ production by ELISA to determine the efficacy of priming. Despite
equal starting numbers, only irradiated tumors were effective in priming DCs for antigen presentation (Fig. 2A, and boiled tumor data not shown). The ability to load mouse DCs with irradiated tumors was confirmed independently in a similar model system using OVA-transfected B16/BL6 melanoma. In both the EG.7 and B16 OVA models, effective priming was reproducible but was not demonstrated in all of the experiments. The optimal DC:tumor ratio for effective priming was 3:1 (data not shown). Whereas irradiated tumor primed DCs stimulated the release of IFN-γ from purified OT-1 CTL, in two of three cases, freeze-thaw lysate/DC vaccines were inhibitory. As shown in Fig. 2B, IFN-γ production from OT-1 CTLs stimulated by OVA peptide was remarkably inhibited by the addition of freeze-thawed tumor. This inhibitory effect was tumor dose dependent and was not overcome by a longer culture period after lysate removal (Fig. 2C and data not shown). In contrast, the addition of whole tumor to cultures containing OVA appeared to augment IFN release, in comparison to cultures containing DCs primed with either OVA or whole tumor cells alone. These data suggest that irradiation is an effective means of DC priming, whereas freeze-thaw tumor lysis inhibits the CTL response to primed DCs.

**Tumor Antigen Presentation by Human DCs.** 624 MEL is a melanoma line expressing the gp100 antigen. 624 MEL was treated by our methods and used to prime DCs derived from an HLA-A2-positive donor. After 24-h incubation, a CTL clone specific for gp100 was added to cultures at a 10:1 CTL:DC ratio, and supernatants were collected at 24 and 48 h. Using 624 MEL at a 3:1 tumor:DC ratio, effective priming was demonstrated in two separate experiments. In one additional experiment, increase in IFN release was only evidenced at 24 h and was not convincingly greater than the CTL/DC control. Only supernatants from DC cultures loaded with irradiated tumor cells demonstrated increased evidence of IFN-γ release in comparison to controls. (Fig. 3).

To demonstrate that this effect was secondary to cross-priming, we performed an analogous set of experiments using the MEL888 melanoma, which expresses GP100 and is HLA-A2 negative. In two separate experiments, T cells stimulated with irradiated MEL888-primed DCs evidenced significantly increased levels of IFN-γ release in comparison to controls. Thus, a total of four separate experiments demonstrated effective priming at the 3:1 tumor:DC ratio. Interestingly, in one experiment, we were able to demonstrate good priming with MEL888, whereas loading with MEL 624 did not stimulate release IFN-γ. This suggests that MEL888 might express higher levels of GP100 than 624 MEL. As were observed in murine studies, effective priming was not observed in all of the experiments. Experiments performed using the 1:1 tumor:DC ratio demonstrated IFN release in one of five experiments, which correlates with our murine data suggesting that a 1:1 tumor:DC ratio is suboptimal for loading.
These data demonstrate that DCs are effectively primed with antigens from irradiated tumors and extends our mouse in vitro findings into an in vitro human tumor model.

**DCs Primed with Irradiated Tumor Cells Effectively Prevent Tumor Outgrowth.** Both the E.G7 murine thymoma and poorly immunogenic SCCVII models were used to correlate in vitro findings with relevant clinical models of disease. Groups of four to five mice were primed by intradermal injection of $1 \times 10^6$ DCs alone, irradiated tumor alone, DCs + irradiated tumor, or DCs + tumor lysates. One group of animals was not primed and served as a no treatment control. Animals received two vaccinations at weekly intervals, and the ratio of tumors:DCs was 3:1. One week after the final vaccination, animals were challenged by local ID injection of $1 \times 10^5$ (E.G7) or $1 \times 10^6$ (SCCVII) tumor cells, and growth was assessed at regular intervals by a blinded observer to determine therapeutic efficacy. In both the E.G7 experiments and the SCCVII model, local tumor outgrowth was inhibited by vaccination with DCs primed with autologous irradiated tumor. In E.G7 experiments three of five animals remained tumor free, whereas 13 of 17 animals did not develop tumors in the SCCVII model. In contrast, in the SCCVII model, 20 of 20 mice in the no treatment group, 13 of 13 mice treated with DCs alone, 4 of 4 mice treated with irradiated tumor alone, and 8 of 8 mice treated with DCs primed with freeze thaw lysates developed tumors. In the SCCVII model, the $P$ for testing no treatment, DCs alone, and DC/FT against DC/XRT were all $P < 0.001$. The $P$ for XRT versus DC/XRT was 0.01. Representative experiments are shown in Fig. 4, A and B. These data demonstrate that irradiated tumor is an effective means of priming DCs for the prevention of primary autologous tumor outgrowth after local challenge. These experiments also serve to validate the relevance of our in vitro studies in a poorly immunogenic solid tumor model.

Subsequent experiments were conducted in a well-established metastases model to determine the efficacy of DCs primed with irradiated tumor for the treatment of established disease. Groups of five animals with 4-day established SCCVII lung metastases were treated with weekly vaccination of autologous irradiated tumor primed DCs $\times 2$. Both survival and lung metastases were monitored to determine the efficacy of therapy. There was no significant difference in survival between animals treated with DC-based vaccines versus untreated controls. Additional maturation of the DCs with anti-CD40 did not enhance therapeutic efficacy. At the time of sacrifice, the number of lung metastases did not differ significantly between groups.
tumors are not effective for the treatment of established disease in this form of therapy provides protective immunity, DCs plus irradiated (data not shown). These data clearly demonstrate that whereas this priming strategies are advantageous in providing many tumor-specific antigens, they are time consuming, expensive, and able to evaluate only single priming parameters. (6) Although the present model relies on the presentation of only one defined antigen (OVA), multiple priming strategies can be assessed within a single assay. Additionally, this study documents the correlation with in vitro findings and the generation of in vivo antitumor reactivity.

Many strategies exist for priming the DC antitumor immune response including peptide loading, freeze-thaw lysis, DC/tumor cell fusion, and DC priming with tumor RNA. (24–26) Whole tumor priming strategies are advantageous in providing many tumor-specific and tumor-associated antigens, which will theoretically decrease the ability of tumors to evade the immune response by down-regulation of a single antigen. Enthusiasm for clinical application of this approach is tempered by uncertainty regarding the optimal means of tumor cell preparation for priming, impurity of fresh tumor cell preparations, the potential for induction of autoimmunity by the presentation of self-antigens, debate regarding the optimal tumor:DC ratios for effective antigen priming, and the lack of evidence for effective management of established disease.

Several methods of whole tumor preparation are effective in priming DCs to elicit an antitumor immune response. Specifically, Fields et al. (6) demonstrated that tumor lysates were capable of priming DCs to stimulate a tumor-specific T-cell response. These tumor lysates served as an effective vaccine both to prevent tumor-specific outgrowth in a protection model and reduce the number of metastases in a therapeutic model. In a relevant clinical model, Nestle et al. (2) used autologous tumor lysate-pulsed DCs for the treatment of stage IV melanoma and demonstrated therapeutic efficacy in 5 of 16 patients. Unlike these previous trials, our studies demonstrate that tumor lysates inhibit peptide-specific CTL activity in vitro in a dose-dependent fashion and do not stimulate protective immunity in tumors of different histological types. The authors wish to stress that the method of lysate preparation used in this study is different from that reported by Fields et al. (6) and may explain the differing results. Other potential explanations for these diverse findings include the use of different tumor strains, alternate vaccination schedules, and the use of DCs at various stages of maturity (2). Importantly, the clinical efficacy of both tumor lysates and fusion for DC priming demonstrates the validity of these approaches.

In the current study, irradiated tumor-primed DCs were the only effective method for generating local protection from tumor outgrowth. The efficacy of irradiated tumor versus other whole tumor strategies likely relates to both the cause/timing of tumor cell death and the means by which tumor antigens obtained by this strategy are presented by DCs. Phenotypic analysis of freeze-thaw-treated tumor cells in our study revealed double-positive expression of PI and annexin V. Positive PI staining implies that the plasma membrane is incompetent (23). This cell lysis is likely induced in the absence of either apoptosis or necrosis as they are characterized in vitro, secondary to the lack of pathological stimuli. In contrast, irradiated cells were phenotypically similar to untreated controls. Because irradiated tumor-primed DCs and irradiated tumors alone never evidenced growth at the vaccine site, it is likely that these cells were functionally nonviable. Previous studies demonstrate that irradiation results primarily in necrotic versus apoptotic tumor cell death (27, 28). Whereas phenotypic analysis of irradiated cells in our study was likely performed too early to document the ultimate means of cells death, it demonstrates that irradiated cells were phenotypically viable at the
time of exposure to DCs and likely were undergoing cell death during vaccine preparation.

Studies by Melcher et al. (18) and Todryk et al. (29) using in vivo immunogenicity assays of cell lines clearly demonstrate that cells killed by necrosis are more immunogenic than those killed by apoptosis. Two recent studies have suggested a potential mechanism, demonstrating that necrosis but not apoptosis induces DC maturation and enhances specific antigen presentation to CD8 T cells (30, 31). Interestingly, these two studies induced necrotic cell death by freeze-thaw lysis, which is likely distinct from necrosis occurring secondary to underlying pathological process because of the inability for rapidly lysed cells to up-regulate stress induced molecules such as HSP (19). Thus, whereas apoptotic bodies are effective in priming APCs in vitro, they likely cannot stimulate DCs to generate an antigen immune response in the absence of additional maturation in vitro. (18) This suggests that one reason for the enhanced antigen efficacy of DCs primed with irradiated tumor is the induction of stress-induced cell death during the period of exposure to DCs in culture.

A second potential explanation for the diverse antigen immune response generated by DCs primed with tumors prepared by different methods is potential variations in the mechanism of antigen presentation. Specifically, studies by Berard et al. (11) clearly demonstrate that monocyte-derived human DCs primed with killed allogeneic melanomas are capable of stimulating naive T cells to recognize shared melanoma antigens. These data suggest that cross-priming, defined as the presentation of foreign antigens on Class I MHC molecules, is an important mechanism in this model system. In addition, DCs primed with irradiated tumor may present antigen by spontaneous tumor DC fusion and bystander effects, in which DCs provide appropriate costimulation for tumor-stimulated T cells secondary to their proximity. These mechanisms are likely of lesser importance in strategies, which use cell lysis, secondary to a failure to maintain cellular integrity.

The critical findings of this paper are that autologous irradiated tumor is an effective means of priming immature DCs to stimulate an effective peptide-specific CTL response in vitro. DCs primed with tumors treated by sequential freeze-thaw lysis inhibited antigen-specific CTL activity in vitro. Effective generation of CTL activity using irradiated tumor/DC in vitro vaccines translated into the induction of protective immunity to primary local tumor challenge in vivo in two different animal models. FACs analysis of irradiated tumor cells by PI and annexin V immediately after treatment demonstrated that these cells were phenotypically viable. This suggests that cells undergoing stress-induced death during the time of exposure to DCs enhances antigen priming. We are currently evaluating whether this protective response is systemic in nature and refining our irradiated tumor-based DC priming strategies in an attempt to stimulate durable therapeutic immunity.

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