

Generation of Effective Antitumor Vaccines Using Photodynamic Therapy¹

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Abstract

Preclinical studies have shown that photodynamic therapy (PDT) of tumors augments the host antitumor immune response. However, the role of the PDT effect on tumor cells as opposed to the host tissues has not been determined. To test the contribution of the direct effects of PDT on tumor cells to the enhanced antitumor response by the host, we examined the immunogenicity of PDT-generated murine tumor cell lysates in a preclinical vaccine model. We found that the PDT-generated tumor cell lysates were potent vaccines and that PDT-generated vaccines are more effective than other modes of creating whole tumor vaccines, *i.e.*, UV or ionizing irradiation, and unlike other traditional vaccines, PDT vaccines do not require coadministration of an adjuvant to be effective. PDT vaccines are tumor specific and appear to induce a cytotoxic T-cell response. We have demonstrated that although both UV and PDT-generated tumor cell lysates are able to induce phenotypic DC maturation, only PDT-generated lysates are able to activate DCs to express IL-12, which is critical to the development of a cellular immune response. Our results show that PDT effects on tumor cells alone are sufficient to generate an antitumor immune response, indicating that the direct tumor effects of PDT play an important role in enhancing that host antitumor immune response. These studies also suggest that in addition to the role of PDT as a therapeutic modality, PDT-generated vaccines may have clinical potential as an adjuvant therapy.

Introduction

PDT³ uses a photoreactive drug or photosensitizer in combination with a specific wavelength of light to kill tumor tissue (reviewed in Ref. 1). Clinical trials have shown a palliation of advanced disease and long-term control of early disease (1). PDT is approved for multiple indications in the United States, Canada, Japan, and 17 countries of the European Union. Numerous preclinical studies have demonstrated that PDT enhances the host antitumor immune response (1, 2), but the mechanisms behind this enhancement are unknown. Among the potential contributing factors are alterations in the tumor microenvironment via stimulation of proinflammatory cytokines and direct effects of PDT on the tumor that increase immunogenicity. Previous studies have shown that PDT stimulates the expression of several inflammatory mediators, including tumor necrosis factor- α , IL-6, and IL-1 (1, 3). However, little is known about the direct effects of PDT on the inherent immunogenicity of the tumor cells. To test these effects we have used PDT to generate tumor cell lysates and used these lysates to inoculate naive animals against subsequent tumor challenge. The studies described in this report indicate that PDT-

generated tumor cell lysates are strongly immunogenic and are effective antitumor vaccines, which function in the absence of adjuvants.

Materials and Methods

Animals and Vaccine Protocol. BALB/cJ (EMT6 tumor host) and DBA/2J (P815 tumor host) mice, obtained pathogen-free from The Jackson Laboratory (Bar Harbor, ME), were used for all of the experiments. Animals were housed in microisolator cages in a laminar flow unit under ambient light. Six to 12-week-old animals were vaccinated intradermally on the right shoulder with 30 μ l of lysate (3×10^5 cell equivalents) or medium control once a week for 4 weeks. The animals were rested a week and then inoculated on the flank with 1×10^4 tumor cells harvested from exponentially growing cultures. Tumor growth was monitored for 90 d; animals were sacrificed when the tumor growth reached 400 mm³ or at 90 d. At least five mice were used for each experimental group.

Generation of Lysates. EMT6 cells were grown in Basal Medium Eagle (BME) supplemented with 15% FBS and antibiotics (all from Life Technologies, Inc., Grand Island, NY); P815 cells were grown in DMEM supplemented with 10% FBS, sodium pyruvate, L-glutamine and antibiotics; Colon 26 cells were grown in RPMI 1640 supplemented with 10% FBS and antibiotics. All of the cells were cultured in a humidified atmosphere of 5% CO₂ in air at 37°C. For PDT-generated lysates, exponentially growing cells were exposed to 2.5 μ g/ml Photofrin (QLT PhotoTherapeutics Inc., Vancouver, British Columbia, Canada) in complete medium for 24 h, followed by exposure to drug-free complete medium for 3 h. Cells were then transferred to serum-free medium (Ham's F12; Life Technologies, Inc.) at 1×10^7 cells/ml and illuminated with 630 nm light via an argon-dye laser system (Spectra Physics, Mountainview, CA) with a dose equivalent to the LD₉₉ (P815 ~ 0.8 J/cm²; EMT6 ~ 1 J/cm²). Treated cells were incubated in a humidified atmosphere of 5% CO₂ at 37°C for 48 h. For UV-generated lysates, P815 cells (1×10^7 /ml) were washed twice in PBS and irradiated with a pair of FS40 sunlamps (290–320 emission spectra). Cells received a total dose of 180 J/m² at 3.8 W/m² (\sim LD₉₉). After treatment, cells were resuspended in Ham's F12 medium and cultured as described above. IR lysates were generated by treatment of the cells with 50 Gy (LD₉₉) of γ -irradiation, followed by a 48 h incubation in Ham's F12 at 1×10^7 cell/ml. LD₉₉ levels were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (4) 48 h after treatment. After the 48 h incubation, cells and supernatants were collected and spun at 800 \times g to clear cell debris and any remaining live cells. The resulting supernatant was collected and frozen at -70°C until use. F/T lysates were generated by subjecting cells (1×10^7 cell/ml Ham's F12) to three F/T cycles, followed by centrifugation at 800 \times g to remove the cell debris. A minimum of two independent lysate preparations by each method was used.

ELISPOT Assay for IFN- γ . Vaccinated and control mice were sacrificed 1 week after the final vaccination. Spleens were harvested, RBC-depleted single cell suspensions were generated, and ELISPOT assays (5, 6) were used to quantitate the numbers of IFN- γ secreting cells. The capture antibody, a rat antimouse IFN- γ monoclonal antibody (R4-6A2; PharMingen, La Jolla, CA) was used at 10 μ g/ml (50 μ l/well) in ester cellulose-bottom plates (Millipore, Bedford, MS). Spleen cells at 5×10^6 cells/ml culture medium (RPMI 1640 supplemented with 10% FBS, L-glutamine, antibiotics, and β -mercaptoethanol) were added to the plate (50 μ l/well). The cells were incubated for 24 h at 37°C without (medium alone) or with stimulation. For stimulation either ConA (1 μ g/ml; positive control), irradiated P815 cells (5×10^5 cells/well; 50 Gy), PDT, UV, or IR-generated lysates (10^5 cell equivalents/well) in a total volume of 50 μ l was added. After the culture period, cells were removed by washing the plate in PBS-Tween (0.05%), and a biotinylated antimouse IFN- γ monoclonal antibody (XMG1.2; PharMingen) was added at 5 μ g/ml (50 μ l/well).

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³ The abbreviations used are: PDT, photodynamic therapy; ConA, Concanavalin A; FBS, fetal bovine serum; IL, interleukin; DC, dendritic cell; ELISPOT, enzyme-linked immunospot; LD₉₉, lethal dose₉₉; F/T, freeze/thaw; HSP, heat shock protein; IR, ionizing irradiation.

The plates were incubated at room temperature for 2 h. The antibody was then removed, and streptavidin-horseradish peroxidase (1:1000; PharMingen) was added. Spots were developed with the substrate AEC (Sigma Chemical Co., St. Louis, MO). Each spot represents an IFN- γ -secreting cell. A minimum of six replicates was performed per sample. Wells containing stimuli alone did not result in spots after the assay.

Chromium (^{51}Cr) Release Assay. Vaccinated and control mice were sacrificed 1 week after the final vaccination. Splens were harvested and RBC-depleted single cell suspensions were generated. Cytolytic activity was measured as described previously (7). Briefly, ^{51}Cr -labeled target cells were incubated with splenocytes at various ratios for 4 h at 37°C. After incubation, supernatants were harvested, and radioactivity was measured by scintillation counting (Packard Instruments Co., Meriden, CT). Results are presented as percentage of specific lysis, which was calculated as follows: percentage of specific lysis = [(observed experimental lysis - the spontaneous lysis observed when target cells were incubated with medium alone)/(maximal lysis observed when target cells alone were incubated with 0.3% Triton X-100 - the spontaneous lysis)] \times 100. The spontaneous lysis was $< 10\%$; three replicates were assayed per sample, and three animals were used per group. Specificity was determined by measuring lysis of a different, H-2 matched tumor cell line. A minimum of six replicates was performed per sample.

DC Studies. Bone marrow-derived DCs (10^6 cells/ml) were isolated from DBA/2 or BALB/c mice as described (8). Briefly, RBC-depleted bone marrow cells were cultured in complete medium (RPMI 1640 supplemented with 10% FBS, L-glutamine, 5 mM β -mercaptoethanol, and antibiotics) containing 20 ng/ml recombinant mouse granulocyte macrophage colony-stimulating factor at 37°C in a humidified atmosphere with 5% CO_2 . The cultures were fed every second day with medium containing fresh granulocyte macrophage colony-stimulating factor. On day 6 of culture, nonadherent cells were removed and washed once in complete medium. Flow cytometry analysis of these cells showed that a majority (70–80%) were MHC class II $^+$ and CD86 $^+$, and were defined as immature DCs (8).

Immature DCs were primed with tumor cell lysates by incubating 10^6 DCs with tumor cell lysates generated from 3×10^6 P815 or EMT6 cells overnight in complete medium at 37°C in a humidified atmosphere with 5% CO_2 . As a positive control DCs were incubated with lipopolysaccharide (1 $\mu\text{g}/\text{ml}$; Sigma Chemical Co.); negative control DCs were incubated with medium alone. After incubation DCs were analyzed for class II and CD86 expression by flow cytometry as described (3). Culture supernatants were harvested and analyzed for IL-12 expression using an ELISA kit specific for murine IL-12 p70 (BioSource, Camarillo, CA). Each experiment was performed a minimum of three times with separate DC preparations.

Statistical Analysis. Survival curves were compared using log rank analyses. All of the other comparisons were done by Student's *t* test. Comparisons with a difference of $P < 0.05$ were considered significant.

Results

PDT Lysates Are Immunogenic and Are Effective Vaccines.

PDT has been shown to enhance the host antitumor immune response (1). To determine whether this enhancement was at least in part a consequence of the effects of PDT on tumor cells, we tested the immunogenicity of tumor cell lysates generated by *in vitro* PDT treatment at a LD_{99} . Naïve mice were vaccinated with PDT-generated tumor cell lysates, F/T lysates, or medium alone as described above. After vaccination the mice were rested 1 week and inoculated with a tumorigenic dose of exponentially growing tumor cells. Survival curves (Fig. 1, A and B) demonstrate the protective nature of the PDT-generated lysates in two tumor model systems, P815 and EMT6. In both cases vaccination with PDT-generated lysates provided significant protection against tumor growth when compared with animals vaccinated with medium alone ($P < 0.0001$ and $P < 0.0099$, respectively). PDT lysates also provided significantly better protection when compared with vaccination with F/T lysates (P815, $P < 0.0045$ and EMT6, $P < 0.0365$); no significant protection was provided by vaccination with lysates generated by F/T cycles when compared with vaccination with medium alone (P815, $P < 0.160$ and EMT6,

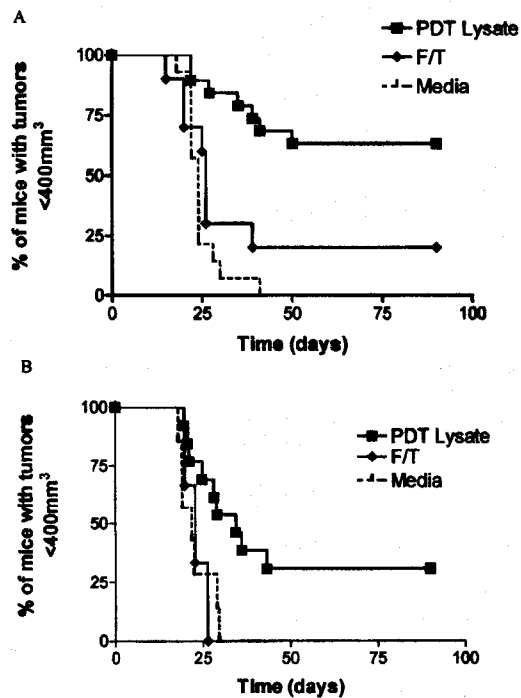


Fig. 1. PDT vaccines provide protection against subsequent tumor challenge in a tumor-specific manner. A, naïve animals were vaccinated with PDT-generated or F/T P815 tumor cell lysates, or with medium alone once a week for 4 weeks. After a week of rest the animals were challenged with 10^4 viable P815 cells, and tumor growth was monitored for 90 days or until the tumors reached 400 mm^3 . Results are reported as the percentage of mice with tumors $< 400 \text{mm}^3$. PDT lysate (\blacksquare) $n = 10$ animals; F/T (\blacklozenge) $n = 10$ animals; medium (broken line) $n = 7$ animals. B, naïve animals were vaccinated with PDT-generated or F/T EMT6 tumor cell lysates, or with medium alone once a week for 4 weeks. After a week of rest the animals were challenged with 10^4 viable EMT6 cells, and tumor growth was monitored for 90 days or until the tumors reached 400 mm^3 . Results are reported as the percentage of mice with tumors $< 400 \text{mm}^3$. PDT lysate (\blacksquare) $n = 13$ animals; F/T (\blacklozenge) $n = 5$ animals; medium (broken line) $n = 7$ animals.

$P < 0.978$). These results demonstrate that PDT directly affected the immunogenicity of P815 and EMT6 tumor cells and that PDT-generated lysates were effective vaccines.

To test the specificity of the tumor vaccines, vaccinated mice were inoculated with an H-2 matched unrelated tumor cell line. Vaccination with PDT-generated EMT6 lysates provided no protection against growth of Colon 26 tumors (data not shown), demonstrating that the PDT-generated vaccines were tumor specific. Similar results were observed with P815 vaccines, and the specificity was confirmed by ELISPOT analysis (see below; Fig. 3A).

PDT Vaccines Provide Better Protection than UV or IR Vaccines. We next compared the effectiveness of PDT-generated vaccines to those generated via treatment with UV or IR. Naïve animals were injected with lysates generated by *in vitro* treatment of P815 cells treated at a LD_{99} level with PDT, UV, or IR as described in "Materials and Methods." As shown in Fig. 2 PDT-generated lysates provide better protection than do those generated by UV or IR (PDT versus UV, $P < 0.05$ and PDT versus IR, $P < 0.04$). No significant differences were observed between the protective effects of UV, IR, or F/T-generated lysates (UV versus IR, $P > 0.79$; UV versus F/T, $P > 0.36$; and IR versus F/T, $P > 0.54$). UV-generated lysates provided significantly better protection than medium alone ($P < 0.008$); lysates generated by IR or F/T were not significantly more protective than medium ($P > 0.11$ and $P > 0.16$, respectively).

PDT Vaccines Induce Tumor-specific IFN- γ -secreting Cells and Increase Cytolytic Activity. The specificity of splenocytes activated by vaccination was examined by ELISPOT analysis (Fig. 3A).

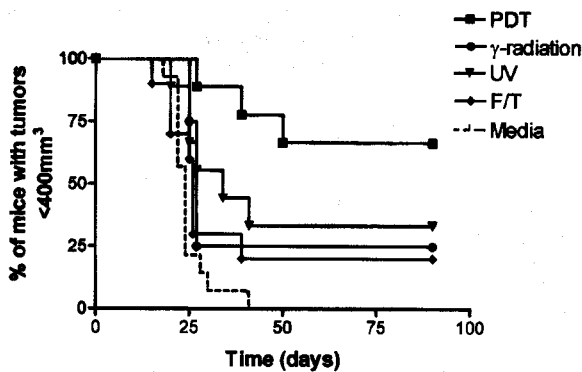


Fig. 2. PDT-generated vaccines are more effective than UV- or IR-generated vaccines. Naïve animals were vaccinated with PDT, F/T, UV, or γ -irradiation-generated P815 tumor cell lysates or with medium alone once a week for 4 weeks. After a week of rest the animals were challenged with 10^4 viable P815 cells, and tumor growth was monitored for 90 days or until the tumors reached 400 mm^3 . Results are reported as the percentage of mice with tumors $< 400 \text{ mm}^3$. PDT lysate (■) $n = 10$ animals; UV lysate (▼) $n = 10$ animals; γ -irradiation lysate (●) $n = 5$ animals; F/T (◆) $n = 10$ animals; medium (broken line) $n = 7$ animals.

Spleen cell preparations isolated from mice vaccinated with lysate preparations contained significant levels of IFN- γ cells when stimulated by either PDT-generated P815 tumor lysates or P815 cells *in vitro*, as compared with spleen cell preparations isolated from mice vaccinated with medium ($P < 0.0001$). In contrast none of the lysate preparations induced splenic cells that responded to *in vitro* stimulation with EMT6 cell lysates, confirming the specificity of the response. Stimulated spleen cell preparations isolated from mice vaccinated with PDT-generated lysates had significantly more IFN- γ secreting spleen cells when compared with stimulated spleen cell preparations isolated from mice vaccinated with either UV (stimulated with PDT P815 lysates, $P < 0.0045$; stimulated with P815 cells, $P < 0.0001$) or F/T lysates (stimulated with PDT P815 lysates, $P < 0.0015$; stimulated with P815 cells, $P < 0.0001$). Spleen cells from mice vaccinated with medium did not contain significant levels of IFN- γ -secreting cells when stimulated *in vitro* with either PDT-generated lysates or P815 cells ($P < 0.59$); spleen cells from all of the groups contained significant levels of IFN- γ -secreting cells when stimulated with ConA ($P < 0.0001$).

To determine whether vaccination with PDT-generated lysates increase cytotoxicity, cytolytic activity present in the spleens of vaccinated mice was measured. As shown in Fig. 3B, PDT- and UV-generated lysates significantly increased cytolytic activity present in the spleens of vaccinated mice when compared with the activity in mice vaccinated with medium alone ($P < 0.0035$ at E:T ratio of 200:1). Vaccination with PDT-generated lysates was significantly better at increasing cytolytic activity when compared with vaccination with either F/T or UV-generated lysates ($P < 0.0045$ at E:T ratio of 200:1).

PDT-generated Lysates Activate DCs. T-cell immunity is initiated by interaction of naïve T cells with mature DCs (9). Immature DCs are potent phagocytes but poor T-cell stimulators (10). Therefore, DC maturation is a critical step in the induction of the immune response. It is possible that a reason PDT-generated lysates are more effective tumor vaccines is that they are better able to stimulate DC maturation than lysates generated by UV, IR, or F/T. To test this hypothesis we examined the effect of various lysates on phenotypic and functional DC maturation. Immature DCs (MHC class II^{lo}, CD86⁻) were isolated from 6-day DBA/2 bone marrow cultures and incubated overnight at a 1:3 ratio with tumor cell lysates generated by PDT, F/T, UV, or IR.

Expression of MHC class II or CD86 was determined by flow cytometry (Fig. 4A). Incubation with both PDT and UV-generated lysates significantly increased the number of mature DCs (class II^{hi}, CD86⁺) when compared with incubation with lysates generated by F/T or IR ($P < 0.003$). Similar results were observed when DCs isolated from BALB/c bone marrow cultures were incubated with PDT or UV-generated EMT6 cells lysates (data not shown).

The functional state of DCs incubated with tumor cell lysates was analyzed by assaying supernatants from tumor cell lysate:DC cultures for the presence of IL-12. IL-12 secretion is a measure of functional DC maturation. Interestingly incubation of immature DCs with PDT-generated lysates induced significantly more IL-12 secretion than did incubation with lysates generated by F/T, UV, or IR (Fig. 4B; $P < 0.014$). IL-12 secretion was not significantly different between cultures containing lysates generated by F/T, UV, or IR ($P > 0.934$), and the amount of IL-12 secreted in these cultures was not significantly different from the amount secreted with DCs incubated with medium alone ($P > 0.582$). None of the lysates themselves contained IL-12 (data not shown). Thus, whereas PDT and UV-generated lysates both induced phenotypic maturation of DCs, only PDT-generated tumor cell lysates induced function maturation.

Discussion

To our knowledge this is the first demonstration of the ability of PDT to enhance tumor cell immunogenicity, the first report of the use of PDT to generate tumor vaccines, and the first report of a crude

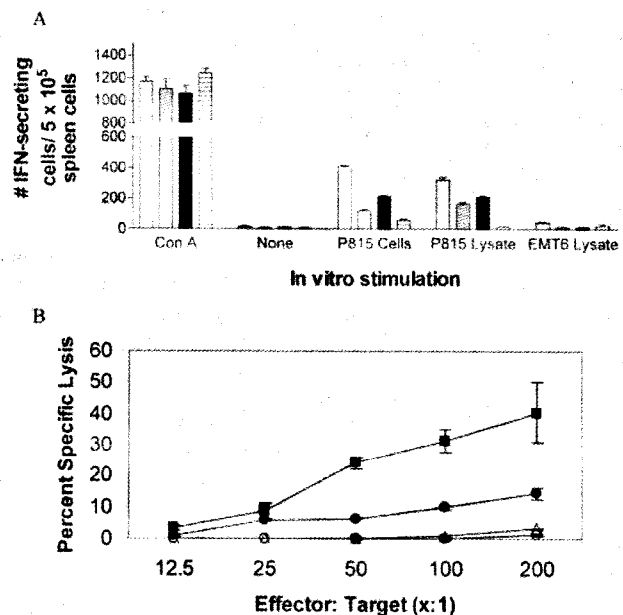


Fig. 3. PDT vaccination stimulates tumor-specific T cells. Naïve animals (three/group) were vaccinated with PDT-, UV-, or F/T-generated P815 tumor cell lysates or with medium alone once a week for 4 weeks. On week 5 the mice were sacrificed, and single cell spleen cell suspensions were generated. A, spleen cells were stimulated with either ConA, P815 PDT-generated lysate, EMT6-generated lysate, or P815 cells or not stimulated (none) for 24 h. After stimulation the number of IFN- γ -secreting spleen cells was determined by ELISPOT analysis as described in "Materials and Methods." Results are reported as a number of IFN- γ -secreting cells per 5×10^5 spleen cells; bars \pm SE. Spleen cells isolated from mice vaccinated with PDT-generated P815 lysates (□); cells isolated from mice vaccinated with F/T generated P815 lysates (▨); cells isolated from mice vaccinated with UV-generated lysates alone (■); cells isolated from mice vaccinated with medium alone (○). B, cytolytic activity was measured as described in "Materials and Methods." Results are reported as percentage of specific lysis of ^{51}Cr -labeled target cells; bars, \pm SE. Cells isolated from mice vaccinated with PDT-generated P815 lysates (■); cells isolated from mice vaccinated with F/T generated P815 lysates (▨); cells isolated from mice vaccinated with UV-generated lysates (●); cells isolated from mice vaccinated with medium alone (○).

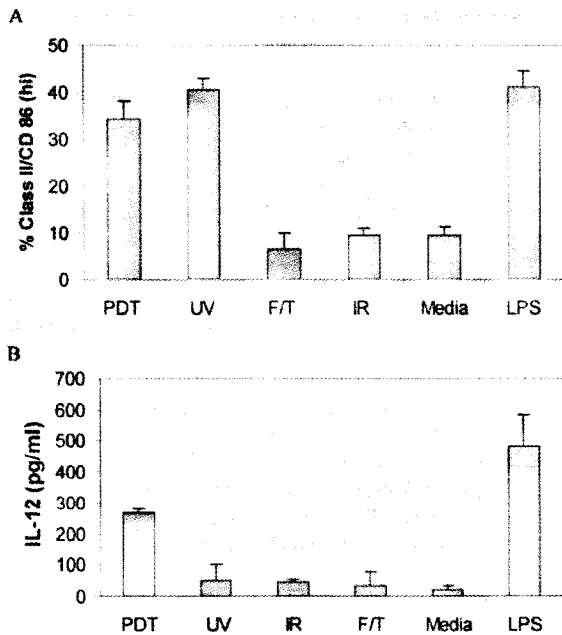


Fig. 4. PDT-generated tumor cell lysates activate DCs. *A*, immature DCs were isolated from DBA/2 bone marrow cultures and incubated overnight with tumor cell lysates as indicated on the X axis at a 1:3 ratio. After incubation the cells were subjected to flow cytometry. Results are reported as percentage of MHC class II^{hi}, CD86⁺ cells in the total population; bars, \pm SE. *B*, supernatants were harvested from DCs/tumor cell lysate cultures described above and assayed for the expression of p70 IL-12 as described in "Materials and Methods." Results are reported as pg/ml IL-12; bars, \pm SE.

tumor vaccine that is functional in the absence of adjuvant. Our studies show that vaccination with PDT-generated tumor cell lysates elicits a tumor-specific immune response as demonstrated by protection against subsequent tumor inoculation, induction of tumoricidal activity in the spleen, and increased numbers of IFN- γ -secreting splenic cells. These studies demonstrate that PDT is able to enhance the inherent immunogenicity of at least some tumor cells.

Our findings on PDT vaccines could contribute greatly to our understanding of how *in situ* PDT amplifies the host immune response. Several preclinical studies (1, 2) and one clinical study (11) have demonstrated that PDT activates the host immune response. Although the mechanism behind this activation is unknown, the response appears to be T-cell dependent (2). A potential mechanism behind the success of PDT-vaccines lies in the ability of PDT-generated tumor cell lysates to stimulate IL-12 production from DCs. IL-12 is required for the development of Th1 (12) and IFN- γ -secreting CD8⁺ (Tc1) cells (13). Th1 cells are critical to the development of a cellular immune response (14), and Tc1 cells promote tumor immunity (15). Incubation of immature DCs with PDT-generated tumor cell lysates *in vitro* stimulated IL-12 production, whereas incubation with UV and IR-generated tumor cell lysates did not. Thus, it is possible that vaccination with PDT lysates activates DCs *in vivo* causing them to secrete IL-12 and initiate a cellular immune response. This hypothesis is supported by the fact that the tumoricidal activity in the spleens of PDT-vaccinated mice is greater than that found in UV- or IR-vaccinated mice. It is also possible that PDT vaccines are effective in the absence of adjuvant because they are able to provide their own adjuvant and directly stimulate the production of IL-12 from DCs. Similar mechanisms may be active after *in situ* PDT, which also causes direct tumor cell death and release of inflammatory mediators. *In situ* PDT may result in the activation of DCs at the tumor site and ultimate stimulation of tumor-specific T cells. Current studies in our laboratory are investigating the fate of immature DCs after *in situ*

PDT. Results from these studies may allow us to enhance the effectiveness of PDT in generating host antitumor immune response and potentially provide for protection against metastases outside the treatment field.

It has been hypothesized that different stimuli are able to trigger different levels of DC activation and that the different activation states trigger different T-cell responses (10). Immature DCs preferentially engulf apoptotic cells (16). PDT induces cell death via both apoptotic and necrotic pathways (17). The dominant mode of cell death after PDT is dependent on the photosensitizer used, the localization of the photosensitizer, and the treatment protocol (18). The PDT treatment protocol used in these studies resulted in a mixture of apoptotic and necrotic cells, whereas UV treatment resulted in apoptotic cell death, and our IR protocol primarily induced necrosis of P815 cells (data not shown). Uptake of apoptotic cells alone is insufficient to induce maturation/activation of DC, and a second signal is required (19). Therefore, it is possible that UV- and IR-generated tumor cell lysates are only able to partially activate DC, which results in less efficient T-cell stimulation. This hypothesis is supported by our findings that UV-generated lysates appear to be only able to partially activate DCs, as evidenced by increased MHC class II and CD86 expression but no increase in IL-12 production, and the results that showed that UV or IR vaccines are not as effective as PDT vaccines at activating tumor-specific IFN- γ -secreting cells and at increasing splenic cytolytic activity. However, partial DC activation by UV lysates appears to be sufficient to induce some T-cell activation, because vaccination with UV-generated lysates were significantly better at inducing tumoricidal activity ($P < 0.0047$) and IFN- γ -secreting cells ($P < 0.0004$) when compared with vaccination with F/T lysates.

The nature of the "activation" factor in PDT-generated tumor cell lysates is unknown, although there are several promising candidates. Inflammatory signals, such as necrotic cells/tissue and HSPs, induce DC maturation/activation (19). The PDT treatment protocol used in this study resulted in the appearance of necrotic cells, and PDT treatment of tumor cells results in the expression of tumor necrosis factor- α , IL-1, and IL-6 (1.3). PDT also stimulates the expression of HSPs (20). A recent report demonstrates that incubation of DCs with HSP60 results in the expression of IL-12 (21).

The EMT6 and P815 tumor models are immunogenic tumor models (22, 23). It will be important to determine how effective PDT vaccines are in the treatment of weakly or nonimmunogenic tumor models. We have preliminary data showing that PDT vaccines are effective in the poorly immunogenic murine alveolar lung carcinoma tumor line. Line 1, and studies by Korbek and Cecic (24) have shown that intratumoral injection of the SCCVII murine line, which is also poorly immunogenic, with PDT-killed cells significantly retards tumor growth.

Although recent studies have focused on the use of genetically engineered cancer vaccines or tumor-associated antigen-primed DCs (25, 26), there is no convincing evidence to date that these vaccines have an overwhelming advantage over crude vaccines (25). In addition, as pointed out recently by Banchereau *et al.* (26), the use of isolated DCs in large-scale immunization is not feasible, and there is a need to develop strategies to activate DCs *in vivo*. These limitations have revitalized interest in the use of "crude" tumor lysates as vaccines, which are generally created by UV or IR of tumor cells and are coinjected with an adjuvant (25, 27). Our studies show that PDT-generated tumor cell lysates are effective antitumor vaccines that are functional in the absence of adjuvant, and direct comparison of PDT-generated vaccines with those prepared by UV or IR indicated that PDT-generated vaccines were superior at eliciting cytolytic activity and providing protection.

The finding that PDT generated tumor cell lysates were effective

antitumor vaccines has potentially broad clinical implications. Not all tumors are amenable to PDT, either because of size or location, and one could conceive of an adjuvant use for PDT vaccines in conjunction with other cancer modalities that do not enhance the host antitumor immune response, such as surgery and/or chemotherapy. Preliminary data from our laboratory has shown that PDT vaccines are effective against established s.c. tumors and lung metastases.

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References

- Dougherty, T. J., Gomer, C. J., Henderson, B. W., Jori, G., Kessel, D., Korbek, M., Moan, J., and Peng, Q. Photodynamic Therapy. *J. Natl. Cancer Inst.*, *90*: 889-905, 1998.
- Korbek, M., and Dougherty, G. J. Photodynamic therapy-mediated immune response against subcutaneous mouse tumors. *Cancer Res.*, *59*: 1941-1946, 1999.
- Gollnick, S. O., Liu, X., Owezarczak, B., Musser, D. A., and Henderson, B. W. Altered expression of interleukin 6 and interleukin 10 as a result of photodynamic therapy *in vivo*. *Cancer Res.*, *57*: 3904-3909, 1997.
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods.*, *65*: 55-63, 1983.
- Czerkinsky, C., Nilsson, C., Nygren, H., Ouchterlony, O., and Tarkowski, A. A solid-phase enzyme-linked immunospot (ELISPOT) assay for enumeration of specific antibody-secreting cells. *J. Immunol. Methods.*, *65*: 109-121, 1983.
- Taguchi, T., McGhee, J. R., Coffman, R. L., Beagley, K. W., Eldridge, J. H., Takatsu, K., and Kiyono, H. Detection of individual mouse splenic T cells producing IFN- γ and IL-5 using the enzyme-linked immunospot (ELISPOT) assay. *J. Immunol. Methods.*, *128*: 65-73, 1990.
- Brichard, V. G., Warmier, G., Van Pel, A., Lucas, S., and Boon, T. Individual differences in the orientation of the cytolytic T cell response against mouse tumor P815. *Eur. J. Immunol.*, *25*: 664-671, 1995.
- Inaba, J., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S., and Steinman, R. M. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.*, *176*: 1693-1702, 1992.
- Lanzavecchi, A., and Sallusto, F. Regulation of T cell immunity by dendritic cells. *Cell*, *106*: 263-266, 2001.
- Mellman, L., and Steinman, R. M. Dendritic cells: specialized and regulated antigen processing machines. *Cell*, *106*: 255-258, 2001.
- Abdel-Hady, E. S., Martin-Hirsch, P., Duggan-Keen, M., Stern, P. L., Moore, J. V., Corbett, G., Kitchener, H. C., and Hampson, I. N. Immunological and viral factors associated with the response of vulval intraepithelial neoplasia to photodynamic therapy. *Cancer Res.*, *61*: 192-196, 2001.
- Macatonia, S. E., Hosken, N. A., Litton, M., Vieira, P., Hsieh, C.-S., Culpepper, J. A., Wysocka, M., Trinchieri, G., Murphy, K. M., and O'Garra, A. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4⁺ T cells. *J. Immunol.*, *154*: 5071-5079, 1995.
- Sad, S., Marcotte, R., and Mosmann, T. R. Cytokine-induced differentiation of precursor mouse CD8⁺ T cells into cytotoxic CD8⁺ T cells secreting Th1 or Th2 cytokines. *Immunity*, *2*: 271-279, 1995.
- O'Garra, A. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity*, *8*: 275-283, 1998.
- Dobrzanski, M. J., Reome, J. B., and Dutton, R. W. Therapeutic effects of tumor reactive type 1 and type 2 CD8⁺ T cell subpopulations in established pulmonary metastases. *J. Immunol.*, *162*: 6671-6680, 1999.
- Albert, M. L., Sauter, B., and Bhardwaj, N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature (Lond.)*, *392*: 86-89, 1998.
- Oleinick, N. L., and Evans, H. H. The photobiology of photodynamic therapy: cellular targets and mechanisms. *Radiat. Res.*, *150* (Suppl. 5): S146-S156, 1998.
- MacDonald, I. J., and Dougherty, T. J. Basic principles of photodynamic therapy. *J. Porphyrins Phthalocyanines*, *5*: 105-129, 2001.
- Nestle, F. O., Banchemau, J., and Hart, D. Dendritic cells: on the move from bench to bedside. *Nat. Med.*, *7*: 761-765, 2001.
- Gomer, C. J., Ryter, S. W., Ferrairo, A., Ryffel, N., Woodard, A., and Fisher, A. M. R. Photodynamic therapy-mediated oxidative stress can induce expression of heat shock proteins. *Cancer Res.*, *56*: 2355-2360, 1996.
- More, S. H., Breloer, M., and von Bonin, A. Eukaryotic heat shock proteins as molecular links in innate and adaptive immune responses: Hsp60-mediated activation of cytotoxic T cells. *Int. Immunol.*, *13*: 1121-1127, 2001.
- Lethe, B., Van den Eynde, B., Van Pel, A., Corradin, G., and Boon, T. Mouse tumor rejection antigens P815A and P815B: two epitopes carried by a single peptide. *Eur. J. Immunol.*, *22*: 2283, 1992.
- Henderson, B. W., Waldow, S. M., Mang, T. S., Potter, W. R., Malone, P. B., and Dougherty, T. J. Tumor destruction and kinetics of tumor cell death in two experimental mouse tumors following photodynamic therapy. *Cancer Res.*, *45*: 572-576, 1985.
- Korbek, M., and Cecic, I. Mechanism of tumor destruction by photodynamic therapy. In: M. S. A. Abdel-Mottaleb and H. S. Nalwa (eds.), *Handbook of Photochemistry and Photobiology*. Stevenson Ranch, CA: American Scientific Publisher, 2002.
- Sinkovics, J. G., and Horvath, J. C. Vaccination against human cancers (Review). *Int. J. Oncol.*, *16*: 81-96, 2000.
- Banchemau, J., Schuler-Thurner, B., Palucka, A. K., and Schuler, G. Dendritic cells as vectors for therapy. *Cell*, *106*: 271-274, 2001.
- Bodey, B., Bodey, B., Jr., Siegel, S. E., and Kaiser, H. E. Failure of cancer vaccines: the significant limitations of their approach to immunotherapy. *Anticancer Res.*, *20*: 2665-2676, 2000.