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Photodynamic Therapy and Anti-Tumor Immunity

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Background and Objectives: Photodynamic therapy (PDT) efficacy appears to be enhanced in the presence of an intact immune system and PDT has been shown to augment anti-tumor immunity. The mechanisms leading to the enhancement of the host immune response to tumor are unclear. Anti-tumor immunity depends upon the presence of activated antigen presenting cells (APCs). These cells are activated by their recognition of components released by pathogens, viruses, dead cells, and the presence of pro-inflammatory mediators. Activated APCs stimulate the generation of cytokine secreting effector cells. Therefore, we have hypothesized that PDT generated inflammatory mediators and components released from tumor cells killed by PDT results in the activation of APCs capable of stimulating effector T-cell proliferation and cytokine secretion.

Study Design/Materials and Methods: To determine the effect of PDT on APCs, tumor draining lymph nodes (TDLNs) of EMT6 or Colo 26 tumor bearing mice were isolated 24 hours after Photofrin-PDT and flow cytometry was used to detect the presence of APCs secreting the T cells stimulatory cytokine, IL-12. APCs were also isolated from TDLNs and used to stimulate T-cell proliferation and secretion of interferon-γ (IFN-γ).

Results: PDT results in an increase in IL-12 expressing APCs in the TDLN. This increase was accompanied by an increase in the ability of APCs isolated from TDLNs of PDT-treated mice to stimulate T-cell proliferation and T-cell secretion of IFN-γ.

Conclusions: Our results indicate that APCs isolated from PDT-treated mice exhibit an enhanced ability to stimulate T-cell proliferation and IFN-γ secretion, suggesting that PDT results in increased APC activity. Lasers Surg. Med. 38:509–515, 2006. © 2006 Wiley-Liss, Inc.

Key words: photodynamic therapy; anti-tumor immunity; antigen presenting cells; IL-12

INTRODUCTION

Photodynamic therapy (PDT) is an established therapy for the treatment of cancer that uses a combination of light and photosensitizing drugs to induce damage to tumor tissue [1]. Photofrin-PDT is approved by the FDA for the treatment of early and late stage non-small cell lung carcinoma and the treatment of high-grade dysplasia associated with Barrett's esophagus [2,3]. Tumor destruction by PDT involves a combination of direct cytotoxicity to the tumor cells and secondary-damaging events caused by vascular shut down and inflammation.

The inflammatory response following PDT has been implicated in the development of anti-tumor immunity [1,4,5]. PDT-enhanced anti-tumor immunity was demonstrated by the studies of Canti et al. showing that mice whose tumors have been eliminated by PDT are resistant to subsequent tumor challenges [6,7] and studies demonstrating that tumor draining lymph node (TDLN) cells isolated from PDT-treated mice are able to transfer tumor immunity to naïve mice [6,8]. The importance of the immune response in long-term tumor control was shown in a series of experiments by Korbelik et al. that demonstrated that PDT treatment, at a dose that was curative in immunocompetent mice, provided only transient tumor control in immuno-deficient SCID, which lack T and B cells [9,10]. This group went on to demonstrate that transfer of splenocytes from PDT-treated animals or naïve animals to SCID mice restored the ability of PDT to control tumor growth long-term [10,11].

Several mechanisms by which PDT-induced inflammation enhances anti-tumor immunity have been suggested but have not been fully explored or proven [12]. Anti-tumor immunity is dependent upon the presence of activated antigen presenting cells (APCs) [13,14]. APCs are activated in response to tissue injury, infection, and cell death [14,15]. Once activated APCs express cell surface molecules needed for T-cell stimulation and cytokines needed for generation of effector T cells [16]. IL-12 is produced primarily by activated APCs (reviewed in [17]) and is critical to the formation of interferon-γ (IFN-γ) secreting effector T cells [18,19]. Our results indicate that APCs isolated from PDT-treated mice express IL-12 and exhibit an enhanced ability to stimulate T-cell proliferation and

Abbreviations used: APC, antigen presenting cell; DC, dendritic cell; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; PDT, photodynamic therapy; Pf, Photofrin; TDLN, tumor draining lymph node.

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IFN-γ secretion, suggesting that PDT results in increased APC activity.

These studies represent one of the first in depth explorations into the mechanisms of PDT enhancement of anti-tumor immunity and support the hypothesis that enhancement of anti-tumor immunity by PDT is due, at least in part, to activation of APCs. The ability of Photofrin-PDT to enhance anti-tumor immunity is intriguing and studies designed to exploit this characteristic in an adjuvant or stand alone treatment modality are underway.

MATERIALS AND METHODS

Animals and Tumor Systems

Pathogen-free BALB/cJ mice were obtained from the Jackson Laboratories (Bar Harbor, ME); SCID mice were obtained from the Roswell Park Cancer Institute Department of Laboratory Animals Resource. Animals were housed in microisolator cages in a laminar flow unit under ambient light. Six to 12-week-old animals were inoculated intradermally on the right or left shoulder with 3×10^5 EMT6 (murine mammary sarcoma) or Colon 26 (murine colon carcinoma) cells harvested from exponentially growing cultures. Tumors were used for experimentation about 10 days after inoculation or when they had reached a size of 5–6 mm in diameter. The Roswell Park Cancer Institute (RPCI) Institutional Animal Care and Use Committee (IACUC) approved all procedures carried out in this study.

In Vivo PDT Treatment

Animals were injected i.v. with Photofrin® (Axcan Scandiapharm, Inc., Birmingham, AL) at 5 mg/kg; 18–24 hours post-injection, tumors were exposed to 630-nm light delivered by an argon dye laser (Spectra Physics, Mountain View, CA) at a total light dose of 135 J/cm^2 at a fluence rate of 75 mW/cm^2. Controls included untreated mice and mice that received Photofrin or light alone. Following PDT, animals were either observed for tumor regrowth, or were euthanized and TDLNs were harvested for specific experimental analyses.

Assessment of Tumor Response

Following treatment, orthogonal diameters of tumors were measured once every 2 days with calipers. The tumor volume, V, was calculated with the formula $V=(lw^2/2)$, were l is the longest axis of the tumor and w is the axis perpendicular to l. The tumors were monitored until they reached a volume greater than 400 mm^3, at which time the mice were sacrificed. Animals were considered cured if they remained tumor free for 60 days after PDT.

Immune Cell Isolation

CD11c$^+$ cells were isolated from tumor draining axillary and brachial lymph nodes (TDLN) of control and experimental tumor bearing mice by magnetic bead technology according to the manufacturer's instructions (Miltenyi Biotec, Auburn, CA). A maximum of two nodes was collected from each mouse and the nodes from each mouse were pooled prior to isolation. Briefly, TDLNs were harvested and incubated in 5 ml of HBSS containing 10 mg collagenase for 30 minutes at 37°C. The suspension was then passed through sieve, washed and suspended to 10^8 cells/ml of 10 mM Hepes-NaOH pH 7.4, 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$. Cells were incubated with CD11c (N418) microbeads for 20 minutes at 4°C, washed and magnetic separation was performed twice. The purity of the resulting cell population was determined by flow cytometry to be greater than 90% CD11c$^+$.

Total T-cell populations (CD3$^+$) were isolated from spleens using a Pan T-cell MACS Isolation Kit (Miltenyi Biotec), which employs negative selection to isolate untouched T cells. Untouched CD4$^+$ T cells were isolated from spleens using a mouse CD4 T-cell isolation kit that employs negative selection and magnetic bead separation (Miltenyi Biotec). Single cell preparations and T-cell isolations were performed in a manner similar to isolation of CD11c cells. The purity of the isolated T-cell populations were determined by flow cytometry to be greater than 90% CD3$^+$ or in the case of CD4$^+$ cells greater than 90% CD3$^+$, CD4$^+$.

Adoptive Transfer

Total T-cell populations were isolated from naïve BALB/cJ spleens and injected i.v. into naïve SCID mice (5×10^6 cells/mouse). Three days post-transfer, the recipient mice were inoculated with EMT6 cells; once the tumors reached treatment size (5–6 mm in diameter) mice were injected with Photofrin and treated with PDT as described above.

Flow Cytometry

Isolated cell populations and infiltrating cell populations present in tumors and tumor draining axillary and brachial lymph nodes (TDLN) before and after PDT were characterized by FACS analysis, using panels of monoclonal antibodies (MAbs) to detect specific cell surface molecules as described previously [20]. Briefly, tumors were disaggregated in 25 ml of HBSS containing 50 mg collagenase, type II (Worthington Biochemical Corp., Freehold, NJ) and 500 mg of bovine serum albumin (BSA) at 37°C for 30 minutes. TDLNs were incubated in 5 ml of HBSS containing 10 mg collagenase at 37°C for 30 minutes. Cells were washed a minimum of three times in PAB buffer (HBSS w/o $Ca^{2+}Mg^{2+}$ containing 5 mg/ml BSA and 1 mg/ml sodium azide). Single cell suspensions in PAB were incubated with MAbs conjugated directly with fluorescein or phycoerythrin, Alexa488 or biotin to quantify cell surface expression of CD4, CD8, CD11c, CD11b, MHC class II (IA^d), CD40, CD80, CD86 (PharMingen, San Diego, CA), or CD205 (Serotec, Raleigh, NC). In cases where biotinylated antibodies were used, streptavidin-cychrome (PharMingen) was added as a detection reagent. Co-stimulatory molecule expression on isolated CD11c$^+$ populations was assessed by two color staining using CD11b and either CD80, CD86, or CD40. Intracellular IL-12 (iIL-12) expression was assessed by three color staining using antibodies specific for CD11c, IA^d, and iIL-12. iIL-12 was measured by following fixation and permeabilization of cells previously stained with MAb specific for cell surface molecules by

incubation with a MAb specific to IL-12 p40/p70 according to the manufacturer's instructions (PharMingen). Appropriate immunoglobulin isotypes were used as controls.

Flow cytometric analysis was performed on a two-laser FACStar Plus (Becton-Dickinson, San Jose, CA) flow cytometer, operating in the ultraviolet (UV) and at 488 nm. Four colors and light scattering properties could be resolved employing 420/20, 530/30, and 575/30 band pass filters and a 640 long pass filter. Data were acquired from a minimum of 20,000 cells, stored in collateral list mode, and analyzed using the WinList processing program (Verity Software House, Inc., Topsham, ME).

T-Cell Proliferation

Increasing numbers of $CD11c^+$ cells isolated from tumor draining axillary and brachial lymph nodes of tumor bearing mice treated with Photofrin or light alone or Photofrin-PDT were added to wells of a 96-well tissue culture plate containing T cells (2×10^5 cells/well) isolated from the spleens of EMT6 or Colon 26 tumor bearing mice. $CD11c^+$: T-cell mixtures were incubated for 48 hours; [^{3}H]-thymidine (1 μCi/well; GE Healthcare Biosciences Corp., Piscataney, NJ) was added during the last 18 hours of culture. Cells were harvested and [^{3}H]-thymidine incorporation was measured with a Packard Matrix 96 beta counter. Corrected counts per minute (cpm) were calculated by subtraction of values obtained in the absence of T cells. T-cell proliferation in the absence of DCs was 62.3 ± 15.5 cpm.

Detection of IFN-γ Production by T Cells

$CD11c^+$ cells isolated from TDLN of control and experimental tumor bearing mice were fixed for 15 minutes at 25°C with 1% paraformaldehyde and washed three times to remove the fixative; residual fixative was "quenched" by incubation for 30 minutes at 25°C in 0.1 mM glycine. Fixation prevents subsequent antigen uptake and processing, facilitating loading of the MHC class II molecules expressed on the $CD11c^+$ cell surface with exogenous peptide. Fixed and washed $CD11c^+$ cells (1×10^4) were cultured in triplicate in round-bottom 96-well plates together with $CD4^+$ T cells (5×10^4) isolated from naïve SFE mice, which are transgenic for an α/β T-cell receptor specific for the hemagglutinin peptide 110-122 (SFERFEIFPKESS) expressed on IA^d [21], in the presence or absence of 2.5 μg/ml peptide. IFN-γ secretion was assessed by ELISA at 24 hours and is reported as μg/10^5 T cells; the assay was performed at least twice and values represent the mean of three independent DC isolations.

Statistical Evaluation

All measured values are presented as means ± SEM (standard error of the mean). The two-tailed Student's *t*-test was used for comparison between groups. *P*-values of 0.05 or less represent statistical significance. For tumor response data analysis, hours-to-event, that is, to 400 mm^3 tumor volume, was calculated for each animal by linearly interpolating between the times just before and after this volume was reached, using log (tumor volume) for the calculations; both tumor volume and hours-to-event calculations were performed using Excel™ (Microsoft, Redmond, WA). Tumor responses between groups were compared using the Kaplan–Meier analysis. Briefly, the calculated hours-to-event data for individual animals were entered in a Prism™ (version 3.0, GraphPad Software, Inc. San Diego, CA) spreadsheet. Prism calculates and graphs event curves (i.e., the fraction of subjects not reaching the events as a function of time) for each group and calculates the group median time-to-event. Event curves were compared by the Prism™ program, using the log-rank test, which calculated a two-tailed *P*-value testing the null hypothesis that the curves were identical.

RESULTS AND DISCUSSION

Long-Term Tumor Control by PDT Is Dependent Upon T Cells

In order to determine which population of cells was required for tumor control following PDT, SCID mice were adoptively transferred with purified $CD3^+$ T cells, inoculated with EMT6 tumor cells, and treated with PDT. As shown in Figure 1, recipient mice treated with PDT were able to control tumor growth while those receiving sham transfers were not ($P<0.002$; SCID+CD3 vs. SCID).

Korbelik et al. [9] and Hendrzak-Henion et al. [7] also demonstrated the importance of T cells in long-term control of tumor growth following PDT. In these studies PDT treatment of EMT6 tumor bearing nude mice, which have minimal T-cell populations and intact B-cell populations, failed to control tumor growth. Further studies have shown that depletion of CD4 and/or CD8 T cells from either immuno-competent animals [7,22] or transferred splenocytes [11] resulted in reduced tumor growth control. The

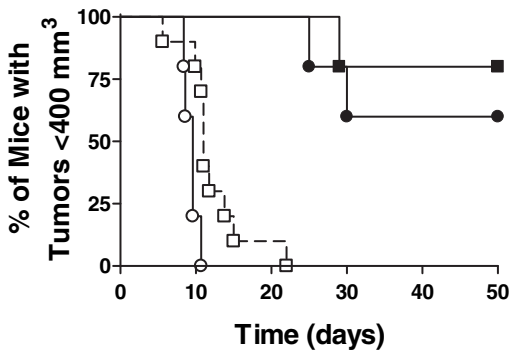

Fig. 1. T cells enhance PDT efficacy. T cells ($CD3^+$ cells) were isolated from naïve BALB/c mice and adoptively transferred into SCID mice; control mice were given sham injections of PBS. Recipient mice were inoculated with EMT6 tumor cells 1 day after T-cell transfer; the resulting tumors were treated with Photofrin-PDT. Tumor growth was monitored for 90 days or until tumors reached a volume of 400 mm^3. Results are reported as the percentage of animals with tumors <400 mm^3. (■) PDT of BALB/c mice; (□) Control BALB/c mice, Photofrin, no light; (●) PDT of T-cell recipient SCID mice; (○) PDT of sham injected mice. Each group contained a minimum of 10 mice.

response of vulval intraepithelial neoplasia patients to PDT can also be correlated to the degree of CD8 T-cell infiltration [23]. Thus, it appears that tumor reactive T cells play a critical role in long-term tumor growth control by PDT.

Photofrin-PDT Increases the Percentage of IL-12 Expressing Antigen Presenting Cells (APCs) in the Tumor Draining Lymph Node (TDLN)

Generation of tumor reactive T cells depends upon the presence of activated APCs that express T-cell stimulatory cell surface molecules (major histocompatibility complex and co-stimulatory molecules) and cytokines such as IL-12 [15]. To determine whether PDT enhanced expression of MHC molecules (IA^d) and IL-12, total TDLN cells from Photofrin-PDT-treated EMT6 tumor bearing mice were examined by three-color flow cytometric analysis for the presence of mature APCs as defined by expression of $CD11c^+$ and MHC class II molecules (IA^{d+}) and for the presence of mature APCs expressing intercellular IL-12 (iIL-12). CD11c is expressed by all subsets of murine dendritic cells [24,25] and some macrophage subsets [26]. Neutrophils also express low levels of CD11c [27] and have been shown to express MHC class II molecules following PDT [28]. Results from a representative experiment are shown in Figure 2A and the combined results of three experiments ($n = 6$ or more mice/group) are shown in Figure 2B, which depicts either the percentage of $CD11c^+$, , IA^{d+} cells (mature APCs, open bars) or $CD11c^+$, IA^{d+}, $iIL\text{-}12^+$ cells (hatched bars) present in TDLN following treatment. These results demonstrate a significant increase in the percentage of mature APCs present in the TDLN following Photofrin-PDT when compared to the percentage of mature APCs present in the TDLN following treatment with light or Photofrin ($P<0.0001$). The percentage of mature APCs expressing iIL-12 also increased following Photofrin-PDT and was significantly greater in

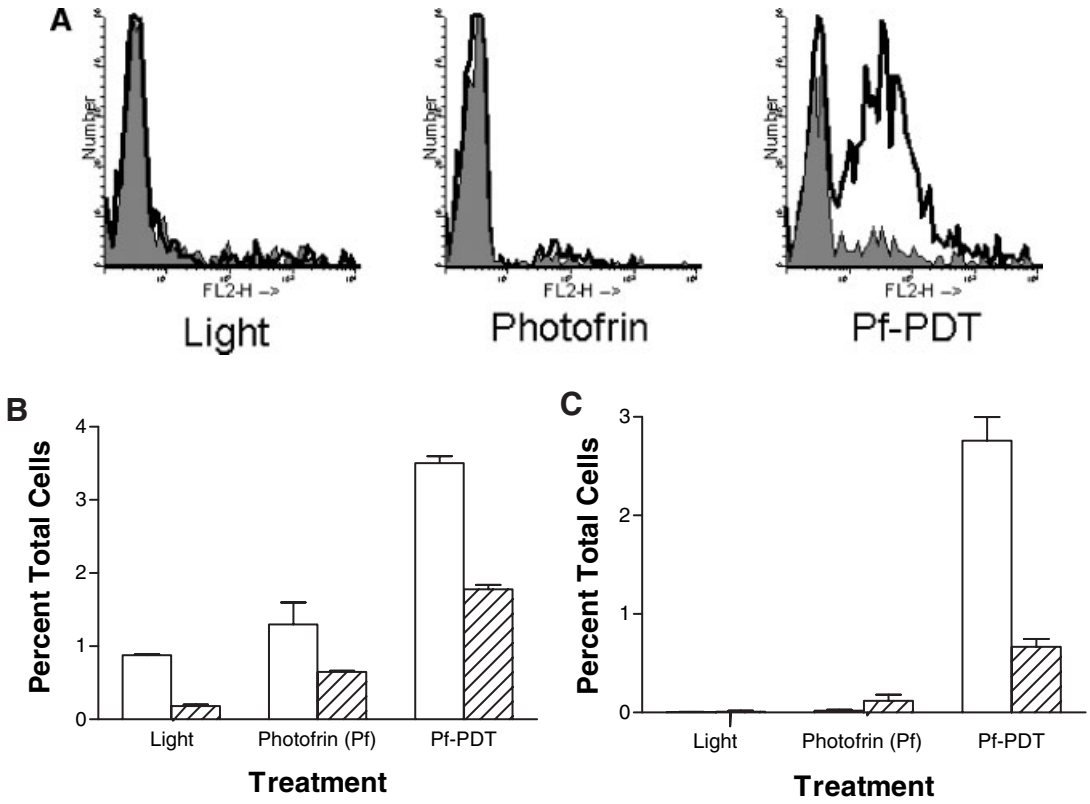

Fig. 2. Photofrin-PDT enhances the percentage of IL-12 expressing DCs in EMT6 and Colo 26 tumor draining lymph nodes (TDLNs). **A**: TDLNs were isolated from EMT6 tumor bearing mice 24 hours post-treatment with light, Photofrin (Pf), or Pf-PDT and cells were analyzed by flow cytometry for co-expression of CD11c, IA^d, and iIL-12. Histograms (event number vs. fluorescence intensity) depicting iIL-12 expression by $CD11c^+$, IA^{d+} cells of a representative experiment are shown. Filled histograms represent staining of the cells with isotype-matched control antibodies; open histograms represent staining with CD11c, IA^d, and iIL-12 specific antibodies. **B**: Results from three independent experiments detailed in (A) are summarized. Open bars represent mature DCs ($CD11c^+$, IA^{d+}); hatched bars represent IL-12 expressing mature DCs. In each graph error bars represent the standard error of the mean; a minimum of six animals was used for each group. **C**: TDLNs were isolated from Colo 26 tumor bearing animals 24 hours after treatment and cells were analyzed by flow cytometry as described in (B). Open bars represent mature DCs ($CD11c^+$, IA^{d+}); hatched bars represent IL-12 expressing mature DCs. In each graph error bars represent the standard error of the mean; a minimum of six animals was used for each group.

the TDLN isolated from PDT-treated mice than from the TDLN of mice treated with light or Photofrin alone (Fig. 2B; $P<0.0003$). Treatment with Photofrin alone slightly elevated the percentage of mature APCs and significantly elevated the percentage of mature APCs expressing iIL-12 (Fig. 2B; $P<0.003$). Photofrin administration has been shown to accumulate in the skin and can result in skin phototoxicity [1]. The increases observed in the percentages of mature APCs and mature APCs expressing iIL-12 following Photofrin administration without subsequent light administration may be a result of inadvertent light exposure, which could result in activation of $CD11c^+$ dendritic cells residing in the skin (Langerhan's cells). Activated Langerhan's cells can secrete IL-12 and migrate to lymph nodes. No significant differences were observed in the percentages of mature APCs or mature APCs expressing iIL-12 found in TDLNs of untreated tumor bearing mice (data not shown) and those treated with light alone (Fig. 2B). To determine the nature of the IL-12 expressing APCs a limited number of samples were co-stained with antibodies specific for CD205. CD205 expression is restricted to dendritic cells and thymic epithelial cells [29,30]. A majority ($>75\%$) of the iIL-12 expressing cells also express CD205 (data not shown).

EMT6 tumors are immunogenic, which may affect the ability of Photofrin-PDT to enhance iIL-12 expression by APCs; therefore, we examined the effect of Photofrin-PDT on the percentage of mature APCs expressing iIL-12 in the TDLN of mice bearing Colo 26 tumors, a less immunogenic tumor model. As shown in Figure 2C, Photofrin-PDT of Colo 26 tumors significantly increased the percentage of mature APCs and mature APCs expressing iIL-12 present in TDLN when compared to the percentage of these cells found in the TDLN of animals treated with Photofrin or light alone ($P<0.001$).

Earlier studies have shown that PDT inhibited the stimulatory ability of DCs and macrophages and reduced the expression of cell surface molecules, including MHC class I and II molecules as well as CD80 and CD86 co-stimulatory molecules (reviewed in [31]). These studies were performed in vitro and involved direct illumination of the cells. Likewise, we failed to detect IL-12 expressing cells in the tumor bed immediately following PDT (data not shown), supporting the idea that direct illumination of these APCs results in a loss or suppression of activity. In contrast our studies examining IL-12 expression, as an indicator of DC activity in the tumor bed 24 hours post-in vivo PDT treatment revealed an increase in activity. It is likely that these IL-12 expressing migrated into the tumor bed following treatment. Several studies have shown that PDT results in rapid infiltration of immune cell types into the tumor bed [4,5].

Photofrin-PDT Enhances the Ability of $CD11c^+$ Cells to Stimulate T-Cell Proliferation and IFN-γ Secretion

To determine whether the increase in activated APCs observed following PDT correlated to an enhanced ability to stimulate T-cell proliferation and polarization, $CD11c^+$ cells were isolated from the TDLNs of EMT6 tumor bearing mice 24 hours after tumor treatment by incubation of single cell suspensions with CD11c microbeads followed by two sequential rounds of magnetic bead separation as described in Materials and Methods. The ability of PDT-activated APCs to stimulated T-cell proliferation was assessed by adding increasing numbers of purified APCs to $CD3^+$ T cells isolated from the spleens of EMT6 or Colon 26 tumor bearing mice. Colon 26 is syngenic with EMT6 and T cells from Colon 26 bearing mice were used as a negative control. The isolated $CD11c^+$ and $CD3^+$ cell populations were greater than 90% pure. APC: T-cell mixtures were incubated for 48 hours and T-cell proliferation was measured by [^{3}H]-thymidine incorporation after 48 hours of incubation. APCs isolated from TDLNs of Photofrin-PDT-treated mice were significantly better able to stimulate proliferation of T cells isolated from EMT6 tumor bearing mice than were APCs isolated from TDLNs of mice treated with Photofrin alone ($P<0.005$ with greater than 50,000 APCs were used; Fig. 3). APCs isolated from TDLNs of PDT-treated EMT6 tumor bearing mice were unable to significantly stimulate proliferation of T cells isolated from Colon 26 tumor bearing mice ($P>0.05$ at all cell concentrations). Thus, the increased accumulation of iIL-12 expressing mature APCs in the TDLN following Photofrin-PDT correlated with an increased ability to stimulate T-cell proliferation in a tumor-specific manner.

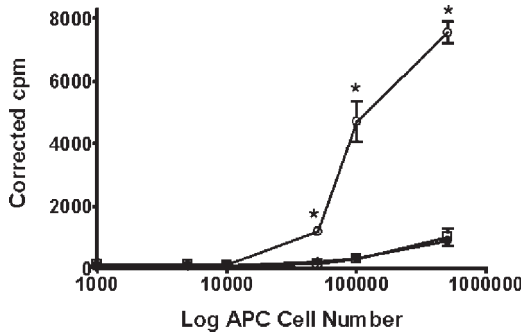

Fig. 3. Stimulation of T-cell proliferation by APCs isolated from Photofrin-PDT-treated mice. APCs ($CD11c^+$) were isolated from TDLNs of EMT6 tumor bearing mice treated with Photofrin or Photofrin-PDT and combined with T cells isolated from EMT6 or Colo 26 tumor bearing mice. Cell mixtures were incubated for 48 hours; T-cell proliferation was determined by [^{3}H]-thymidine uptake. Results are presented as corrected cpm versus log APC number. Corrected counts per minute (cpm) were calculated as described in Materials and Methods. Circles represent stimulation by APCs isolated from PDT-treated mice and square symbols represent stimulation by APSCs isolated from Photofrin-treated mice. Open symbols represent stimulation of T cells isolated from EMT6 tumor bearing mice; closed symbols represent T cells isolated from Colon 26; six animals were used for each group and assays were performed in at least quadruplicate. Asterisks (*) indicate significance over controls ($P<0.005$).

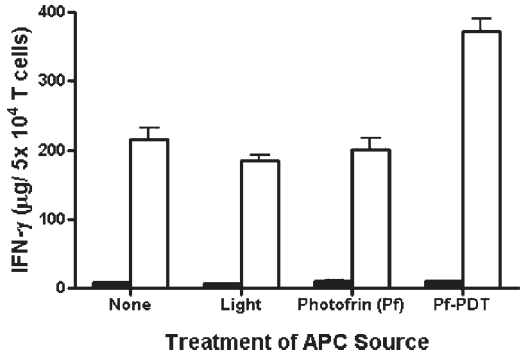

Fig. 4. Induction of IFN-γ secretion from SFE transgenic T cells by APCs isolated from TDLN 24 hours after PDT treatment. CD11c expressing cells were isolated from TDLNs of tumor bearing untreated mice, mice treated with light or Photofrin alone and mice treated with Photofrin-PDT. Isolated cells (10^4 cells) were admixed with $CD4^+$ cells (5×10^4 cells) purified from SFE transgenic mice in the presence (filled bars) or absence (open bars) of SFE peptide and incubated for 24 hours. IFN-γ secretion was measured by ELISA. Results are presented as $\mu g/5\times10^4$ T cells. Error bars represent SEM. APCs isolated from PDT-treated animals resulted in significantly more IFN-γ secretion than APCs isolated from control animals. Four animals were used for each group and assays were performed in at least quadruplicate.

The antibody used in our study to detect IL-12 recognizes the IL-12p40 subunit of the active IL-12p70 heterodimer; this subunit is also present in the cytokine IL-23 [32] and can be secreted as a homodimer that inhibits IL-12p70 activity [32]. Activated APCs also express IL-23 [32]; therefore, it is possible that some of the cells that we determined to be expressing IL-12 could also be expressing either IL-12p40 homodimers or IL-23 as opposed to active IL-12. IL-12 drives naïve T cells to secrete IFN-γ [18,19]; in contrast IL-23 does not promote IFN-γ secretion [33].

To determine whether PDT-activated APCs could stimulate T cells to secrete IFN-γ, purified APCs from TDLNs of EMT6 tumor bearing mice that were either untreated or treated with Photofrin or light alone or Photofrin-PDT were admixed with $CD4^+$ T cells isolated from SFE TcR transgenic mice in the presence or absence of SFE peptide. Cell mixtures were incubated for 24 hours and the resulting supernatant was analyzed for IFN-γ expression (Fig. 4). $CD11c^+$ cells isolated from TDLNs of PDT-treated animals stimulated significantly more IFN-γ secretion from SFE T cells than $CD11c^+$ cells isolated from TDLNs of untreated tumor bearing animals or animals treated with either light or Photofrin alone ($P<0.02$). Minimal levels of IFN-γ were detected in supernatants from APC: T-cell mixtures incubated in the absence of peptide. The enhanced ability of APCs isolated from the TDLNs of PDT-treated mice to stimulate T-cell secretion of IFN-γ suggests that these cells secrete IL-12 rather than inhibitory IL-12p40 homodimers or IL-23.

CONCLUSION

This report represents one of the first studies into the mechanisms of PDT enhancement of the host anti-tumor immune response and shows that in situ PDT leads to an increase in APC activation and ability to stimulate T-cell proliferation and polarization. The mechanism by which PDT enhances APC activation is unclear; however, APC activation in pathogenic and tumor settings has been shown to depend upon the presence "danger signals" [34–36]. Most of the APC activating danger signals identified to date are exogenous and include bacterial, viral, and fungal products [37]. Stress proteins, uric acid, and dying cells have been identified as endogenous mediators [14,38,39]. These mediators are induced by PDT treatment of tumors and have been implicated in the induction of anti-tumor immunity by PDT [1,12,40,41]. Previous studies using have shown that addition of APC enhancing adjuvants or cytokines can enhance PDT efficacy [42–45] and that induction of APC maturation and activation correlates with the ability of PDT generated vaccines to provide protection against subsequent tumor challenge [46,47]. The ability of PDT to enhance anti-tumor immunity is intriguing and suggests that PDT treatment of local tumors may lead to control of distant disease and metastases.

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