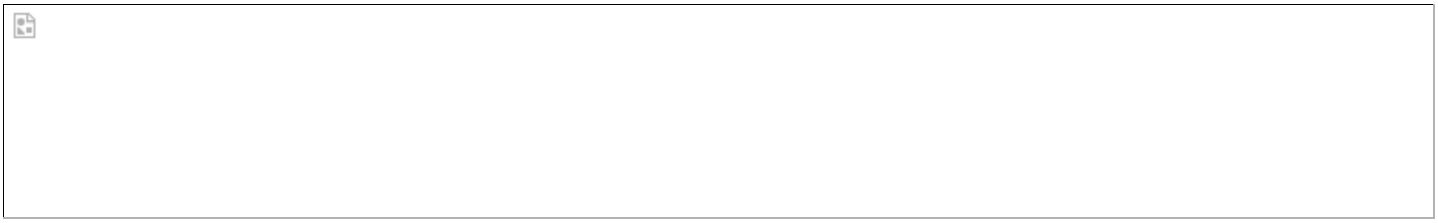

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Interaction Between Photodynamic Therapy and BCG Immunotherapy Responsible for the Reduced Recurrence of Treated Mouse Tumors¶

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ABSTRACT

Subcutaneous mouse EMT6 tumors were treated by individual or combined regimens of a single Bacillus Calmette–Guérin (BCG) vaccine administration and photodynamic therapy (PDT). Six clinically relevant photosensitizers characterized by different action mechanisms were used: Photofrin, benzoporphyrin derivative, tetra(*m*-hydroxyphenyl)chlorin (foscan), mono-L-aspartyl-chlorin *e*6, lutetium texaphyrin or zinc phthalocyanine. Irrespective of the type of photosensitizer used, the optimized BCG protocols improved the cure rate of PDT-treated tumors. This indicates that the interaction does not take place during the early phase of tumor ablation but at later events involved in preventing tumor recurrence. Beneficial effects on tumor cure were observed even when the BCG injection was delayed to 7 days after PDT. The accumulation of activated myeloid cells that markedly increases in tumors treated by Photofrin-based PDT was not additionally affected by BCG treatment. However, the incidence of immune memory T cells in tumor-draining lymph nodes that almost doubled at 6 days after Photofrin-PDT further increased close to three-fold with adjuvant BCG. This suggests that BCG immunotherapy amplifies the T-lymphocyte–mediated immune response against PDT-treated tumors. Since both these modalities are established for the treatment of superficial bladder carcinomas, use of their combination for this condition should be clinically tested.

INTRODUCTION Return to TOC

The mechanism of eradication of solid cancers by photodynamic therapy (PDT)[†] is based on a complex interaction of antitumor effects that have both tumor-localized and systemic character (1–3). From the basic insult inflicted by photodynamic treatment, which is a form of oxidative stress, events are triggered that not only affect cells that have sustained phototoxic damage but also involve a massive release of powerful mediators soliciting a strong reaction from the tumor host. The cells react to the formation of phototoxic lesion by activating signal transduction pathways associated primarily with stress protein regulation (1,4,5). The induction of these physiological signaling cascades results in the downstream activation of not only genes encoding various types of stress proteins but also transcription factors responsible for the expression of numerous immunologically important genes (1). The products of these genes could be responsible for the initiation and propagation of PDT-induced inflammatory/immune reaction (1,5). On the other hand, phospholipid fragments and arachidonic acid metabolites, whose release is caused by photooxidative lesions of cell membrane lipids as well as the induced complement activation, together with the release of histamine, various cytokines and chemokines and other powerful mediators instigate and amplify acute inflammatory processes (3,6). Thus, the treatment of tumors by PDT elicits a strong acute inflammatory reaction associated with a rapid and massive invasion of neutrophils and other myeloid effector cells into the targeted lesion (7,8). The activity of neutrophils and macrophages was found to contribute to the therapeutic outcome of PDT (9,10), which presumably reflects not only on their contribution in the form of direct cytolytic activity against tumor cells but also on the development of cancer-specific immunity (1,3).

Using techniques of bone marrow transplantation and adoptive splenocyte/T cell transfer between immunocompetent and immunodeficient mice, as well as specific depletion of $CD4^+$ and $CD8^+$ cells, we have demonstrated that lymphoid cell activity is required for the PDT-mediated cure of mouse EMT6 mammary sarcomas (11,12). Recovery of immune memory cells from distant lymphoid sites confirmed the induction of a long-lasting systemic immunity raised against even poorly immunogenic tumors (12). This inflammatory/immune character of PDT makes it particularly suitable for being combined with various forms of immunotherapy that can effectively improve the cure rates of treated tumors (3). Among immunotherapy treatments shown to be effective in conjunction with PDT, in addition to adoptive transfer protocols (13,14) and the use of different cytokines or other immune-specific agents (3), are a variety of bacterial vaccines serving as nonspecific enhancers of immune response. With respect to the latter, the beneficial effect on PDT-mediated tumor response was reported for adjuvant treatments with the Bacillus Calmette–Guérin (BCG) vaccine (15,16), mycobacterial cell wall extract (16) and *Corynebacterium parvum* vaccine (17). The BCG vaccine is of special interest as it currently remains the only regulatory approved and standardly used clinical immunotherapy agent with intravesical BCG serving as the most effective treatment for recurrent carcinoma *in situ* and superficial transitional cell carcinoma of the urinary bladder (18). Since PDT has also been used successfully for treating these lesions (19,20) the already demonstrated interaction between the antitumor effects of PDT and BCG (15,16) deserves more detailed evaluation in preclinical and perhaps also clinical studies.

The mechanism of antitumor action of BCG is still under investigation. It is clear that the inflammatory reaction it evokes at the treated site is associated with the development of immune rejection of treated cancer (21). Cytokines and activated lymphocytes were detected in the urine of bladder cancer patients after instillation of BCG (22–24), while persistent infiltrates of immunocompetent cells were found at the treated site (25). However, these observations may reflect solely on the immunological reaction against mycobacterium virulence. The effects implicated in specific antitumoral action of BCG are the activation of macrophages and dendritic cells and associated enhancement of tumor antigen presentation (26,27) as well as the activation of T lymphocytes or lymphoid killer cells (24,28–30).

The aim of the present study was to further characterize and elucidate the nature of the interaction of optimized protocols of adjuvant BCG immunotherapy with antitumor effects of PDT. Because relatively large numbers of tumors/mice were required with extended monitoring for tumor recurrence, in this phase of investigation we opted to use a subcutaneous PDT well-characterized model instead of the clinically more comparable orthotopic (intravesical) tumor model.

MATERIALS AND METHODS Return to TOC

Tumor model. The tumor model used in this study was a mammary sarcoma EMT6, which was maintained in syngeneic BALB/c mice by biweekly intramuscular transplantation. Subcutaneous inoculation of 1×10^6 cells into the lower dorsal region of 7–9 week old female mice was used for preparing the tumors for the experiments (31). They were treated about 1 week after implantation when they reached a largest diameter of 6–8 mm. The experimental procedures were approved by the Animal Care Committee of the University of British Columbia.

Photosensitizers. Photosensitizers Photofrin (porfimer sodium), benzoporphyrin derivative monoacid (BPD, lyposomal preparation veterporfin) and zinc phthalocyanine (ZnPc, lyposomal preparation CGP 55 847) were provided by QLT PhotoTherapeutics Inc. (Vancouver, B.C., Canada). The photosensitizers tetra(*m*-hydroxyphenyl)chlorin (foscan) (*m*THPC) and lutetium texaphyrin (Lu-Tex), (PCI-0123, 2.2 mg/mL in 5% mannitol) were provided by Scotia Pharmaceuticals Ltd. (London, UK) and Pharmacyclics Inc. (Sunnyvale, CA), respectively. A solvent mixture ethanol:polyethylene glycol 400:water 2:3:5 (vol/vol/vol) was used for dissolving and delivering *m*THPC. We synthesized the photosensitizer mono-L-aspartyl-chlorin *e*6 (NPe6) from chlorin *e*6 purchased from Porphyrin Products (Logan, UT); all reactions were performed in the dark or using light-shielded glassware. Briefly, chlorin *e*6 was treated with *O*-(*N*-succinimidyl)-*N,N,N′N′*-tetramethyluronium tetrafluoroborate and triethylamine in freshly distilled dry dimethylformamide in a dry atmosphere, which gave a mixture of *N*-hydroxysuccinimidyl esters. Next, di-*t*-butyl aspartate and triethylamine were added to yield a mixture of di-*t*-butyl aspartate derivatized chlorins, which were then separated by chromatography on silica/(CH_2Cl_2:MeOH), and the desired mono-*t*-butyl aspartate derivative was purified by repeated chromatography. The final product was obtained by stirring in neat trifluoroacetic acid (TFA) with subsequent removal of the TFA under reduced pressure and extraction of the chlorin into ethyl acetate from an acetic acid/dilute aqueous ammonium acetate mixture. When necessary, further purification was achieved by reverse phase column chromatography (Bakerbond WP-Octadecyl media) eluting with MeOH:H_2O:CH_3CN:TFA (2:1:1:0.01). The purity of this compound was controlled by reverse phase thin-layer chromatography and high-performance liquid chromatography.

PDT protocols. For photodynamic light treatment, the mice were restrained unanesthetized in lead holders exposing their backs. The light was delivered from a 1 kW xenon bulb equipped tunable light source (model A5000 from Photon Technology International Inc., Monmouth Junction, NJ) through a 5 mm core diameter liquid light guide 2000A (Luminex, Munich, Germany). The wavelengths used were 630, 652, 664, 671, 690 and 732 nm ± 10 nm for Photofrin, *m*THPC, NPe6, ZnPc, BPD and Lu-Tex, respectively. The fluence rate with Lu-Tex was 60–70 mW/cm^2 and 70–90 mW/cm^2 with other photosensitizers. All photosensitizers were administered intravenously, BPD and Lu-Tex at 3 h, NPe6 at 4 h, Photofrin and mTHPC at 24 h and ZnPc at 48 h before light treatment.

BCG therapy. The BCG vaccine (PACIS^{MD}, IAF BioVac Inc., Laval, Quebec, Canada) freshly reconstituted from liophylizate was used and administrated at 1×10^7 colony-forming units (CFU) per mouse. In most experiments, BCG was injected subtumorally (s.t.). In cases when BCG treatment was delayed to 7 days after PDT (tumors not palpable), it was injected peritumorally. Freund's incomplete adjuvant (FIA) (Sigma Chemical Co., St. Louis, MO) was injected s.t. at 0.1 mL per mouse.

Tumor response evaluation. Tumor response to therapy was evaluated by monitoring the mice for signs of tumor growth every second day up to 90 days after treatment, which qualified as a cure if there was no evidence of growth by that time. Each treatment group consisted of eight mice. In the case of BCG-only treatment changes in tumor volumes were determined by measuring with a caliper the lesion's three orthogonal diameters (four mice per treatment group).

Flow cytometry analysis. The analysis of cell suspensions obtained from EMT6 tumors or their draining

(inguinal) lymph nodes was performed by flow cytometry. Tumor tissue was minced and dissociated into single-cell suspension using a mixture of collagenase, dispase and deoxyribonuclease as described in detail elsewhere (32), while lymph nodes were teased apart and residing cells released using a surgical forceps. Remaining tissue fragments were, in both cases, removed by filtering through a 50 μm nylon mesh and the suspensions aliquoted into samples containing 0.5–1 × 10^6 cells. These samples were then stained with different monoclonal antibodies conjugated with fluorescent chromophores (fluorescein isothiocyanate, Phyco or CyCrome) that were purchased from PharMingen (San Diego, CA), except for the antibodies against mouse F4/80 and DEC-205 antigens obtained from Cedarlane Laboratories Ltd. (Hornby, Ontario, Canada). The staining (20 min on ice) and the subsequent flow cytometry using a Coulter Epics Elite ESP apparatus from Coulter Electronics (Hialeah, FL) were performed employing standard procedures with appropriate staining controls (7,31). Using the antibody to mouse myeloid differentiation antigen Gr-1 (Ly-6G), the main populations in EMT6 tumors were identified as malignant cells (Gr-1^{neg}) and myeloid cells (Gr-1^{pos}), which were further discriminated as macrophages (Gr-1^{med}) and neutrophils (Gr-1^{high}). The antibodies to mouse membrane antigens CD11b and CD62L were used for determining the expression of adhesion molecules Mac-1 integrin and L-selectin. The antibodies used for the analysis of lymph node cells were against mouse CD3 (T lymphocytes), CD4 (helper T lymphocytes), CD8 (cytotoxic T lymphocytes), CD45R (B lymphocytes), F4/80 (macrophages), CD11c and DEC-205 (dendritic cells), CD44 and CD45RB (immune memory cells). Each treatment group consisted of four mice.

Statistical analysis. Statistical analysis of tumor recurrence was based on the log-rank test, while the other results were evaluated using Student's *t*-test.

RESULTS Return to TOC

FIA combined with PDT

A possibility that the therapeutic gain obtained with BCG and other bacterial vaccines used in conjunction with PDT results from nonspecific proinflammatory effects of the vaccines was tested by determining the effect of FIA on PDT response. This adjuvant is an oil emulsion known to be a classical inducer of localized inflammation (33). Similar to the BCG treatment, the subtumoral injection of FIA induces a strong localized edema that persists for several days. However, this treatment performed immediately after PDT light exposure produced no therapeutic gain with established subcutaneous EMT6 tumors treated by *m*THPC-based PDT (Fig. 1).

Optimization of PDT plus BCG protocols

In order to understand better the nature of interaction between the BCG-induced response and the antitumor action of PDT, we undertook to identify optimal conditions for the combined treatment with these two modalities. The details of BCG treatment optimization shown are representative examples obtained with one of the photosensitizers, but they were also validated with other photosensitizers examined in this study. The influence of sequencing the BCG and PDT treatments is shown in Fig. 2. Both pre- and post-PDT treatments with BCG were effective in reducing the incidence of tumor recurrence observed following the initial complete ablation induced by *m*THPC-based PDT, and in increasing the percentage of tumor cures (defined as no evidence of palpable lesions at 90 days after therapy). Subtumoral BCG injections performed immediately after photodynamic light exposure appeared more beneficial than the reverse order of these two treatments, though the difference between these two schedules has not reached statistical significance. When used alone, BCG given as a single subtumoral injection (1 × 10^7 CFU) was not successful in tumor eradication. On the other hand, tumor volume analysis (lower panel in Fig. 2) indicates that BCG temporarily delayed the growth of subcutaneous EMT6 tumors, though the effect was statistically significant only at 7 days after treatment. Hence, this tumor model appears to be responsive to BCG. Many tumors, particularly those that are poorly immunogenic, are not responsive to BCG therapy (34).

The antitumor effect of local BCG treatment is in most cases more effective than systemic BCG administration (35). This also pertains to the use of this vaccine in combination with PDT, as exemplified by the markedly improved response of EMT6 tumors to BPD-based PDT performed in conjunction with tumor-localized injection, but not with intraperitoneal injection, of BCG (Fig. 3 ).

Use of different photosensitizers

The results of BCG treatment combined with PDT based on four different photosensitizers are also shown in Fig. 3  . In addition to BPD are included NPe6, Lu-Tex and ZnPc. The drug–light combinations used in all the cases were chosen to achieve a strong tumor response but very low level, if any, of permanent tumor cures. Since the potency, delivery capacities and other properties of these photosensitizers vary, it was necessary to use different light fluences, illumination wavelengths and drug–light intervals in these experiments. It can be seen that the curative outcome of PDT with these various photosensitizers as well as with *m*THPC (Fig. 2 ) and Photofrin (shown in Fig. 4 ) is improved in all the cases by the combined BCG treatment. In contrast to the other five tested photosenstizers, treatment with ZnPc-PDT does not produce early tumor ablation, reflecting the absence of pervasive early stage vascular damage with this photosensitizer (36). About one-fourth of the tumors treated with the chosen dose of ZnPc-PDT alone became impalpable 10 days after PDT but all of them showed recurrence within the next 2 weeks (Fig. 3 ). The subtumoral administration of BCG performed immediately after photodynamic light treatment increased the rate of complete tumor remission to 50%, and none of these tumors showed recurrence for up to 90 days after therapy.

Delayed BCG treatment

Although scheduling the BCG treatment after PDT is optimal for the beneficial effect of this vaccine on the tumor response to PDT, the interaction of these two modalities is not strictly dependent on their delivery within a short time interval. As shown in Fig. 4  , tumor-localized administration of BCG delayed to 1 week after PDT was still effective in increasing the curative outcome of Photofrin-based PDT. The therapeutic benefit obtained with the protocol involving BCG administered immediately after Photofrin-based PDT with the same tumor model was reported earlier (16).

Role of myeloid and lymphoid host cells

The therapeutic potential of BCG is based on its immunomodulating properties (28,29) that could be responsible for the therapeutic gain observed when this agent is used in conjunction with PDT. To investigate this, we examined the effects of PDT, BCG and their combination on the activity of myeloid and lymphoid populations in treated tumors and tumor-draining lymph nodes. At this stage, we chose Photofrin as the representative photosensitizer because of its status as a clinically established agent. Flow cytometry–based analysis of cellular populations found in EMT6 tumors excised 21 h after treatment showed, in addition to malignant cells, a relatively large content of associated myeloid populations (monocytes/macrophages and neutrophils), as already reported for the same tumor model treated with Photofrin- or *m*THPC-based PDT (8,16). These cells were identified in this experiment by positive staining for the common myeloid marker Gr-1. In contrast, neither nontreated nor treated tumors analyzed at 21 h after treatment showed notable accumulation of lymphoid populations.

The myeloid cell content increased from 46 ± 3% (standard deviation) found in nontreated EMT6 tumors to 73 ± 14% in BCG-treated and to 85 ± 7% in Photofrin-PDT–treated tumors. Following the combined treatment (PDT + BCG), the average tumor content of myeloid populations rose to 91 ± 6%, a value that was not statistically different from that in PDT-only–treated tumors. The majority of neutrophils and macrophages accumulated in PDT-treated tumors were activated, as revealed by the strongly upregulated expression of Mac-1 and L-selectin adhesion molecules (increased staining with anti-mouse CD11b and CD62L antibodies) (37–39) but this characteristic was not further affected by adding BCG treatment (data not shown).

Since Photorin-based PDT results in rapid tumor ablation, the analysis of lymphoid cell activity that develops gradually during a several day period after PDT treatment, requires a different experimental approach than for myeloid populations. This prompted us to examine the cellular content and activity in tumor-draining lymph nodes that were excised 6 days after therapy. The majority of cells found at this site in mice bearing nontreated EMT6 tumors were T lymphocytes (69 ± 5%), the second largest population were B cells (17 ± 5%), while the incidence of macrophages and dendritic cells were 7–8% each (Fig. 5  , pie insets). The treatment of tumors with PDT as well as with the PDT + BCG combination induced no significant changes in the incidence of T and B cells, macrophages and dendritic cells in tumor-draining lymph nodes. The average values for B cell content in these treatment groups was somewhat higher, but the difference was not statistically significant. However, the cellular content in lymph nodes draining BCG-treated tumors was characterized by a greater than two-fold increase in the level of B lymphocytes (to 44 ± 6%), which is in accordance with the known humoral response elicited by this agent resulting in the production of antibodies against BCG (40).

Immune memory cells within T lymphocyte population can be identified by the expression of two membrane molecules, the cell adhesion receptor CD44 (upregulated) and glycoprotein CD45RB (diminished expression) (41). The latter is an exon B–dependent epitope of the leukocyte common antigen CD45. Since the EMT6 tumor model is relatively immunogenic, it was not unexpected to find that about 10% of T cells in the lymph nodes draining nontreated tumors have characteristics of memory cells (Fig. 5 ). However, the incidence of memory cells in this population almost doubled at 6 days following PDT, which confirms the earlier findings on the induction of immune response by this treatment modality (12,42). A similar but smaller increase was registered with samples from BCG-only–treated mice, which, nonetheless, was not statistically significant. The combined treatment of PDT and BCG appears highly effective in inducing the generation of immune memory cells, since the incidence of these cells found in lymph node–residing T cells increased almost three-fold, 6 days after therapy. This was a statistically significant rise compared to the immune memory cell level induced by PDT alone ($P < 0.005$).

DISCUSSION Return to TOC

The present comprehensive analysis clearly demonstrates that BCG (given as a single injection) is a highly effective adjuvant to PDT, as it markedly improves the therapeutic outcome of tumor treatment. The optimized protocols for the combined therapy require tumor-localized BCG administration given after PDT, but the reverse order is also effective. Our investigation has produced no clear evidence that the adjuvant effect of BCG would be substantially better with some and less pronounced with other photosensitizers among those used for PDT in this study. A similar conclusion was drawn from a related study of the effect of PDT combined with mycobacterium cell wall treatment (16). Since the level of effectiveness of BCG treatment is difficult to control and maintain, we concluded that it is not reliable to quantify and compare (generally similar) therapeutic enhancement ratios obtained with BCG used with different photosensitizers. Variations in the viability of the attenuated bacteria from one vaccine preparation to the other, together with variability in the individual host reaction to indirect nonspecific immunomodulatory stimulus of BCG are well recognized (43). These are the most likely reasons for the experimental variation in the magnitude of tumor response with this agent that we observed in the course of our studies.

Of particular interest is the finding that the choice of the photosensitizer for the combined treatment with BCG is not of critical importance. Photofrin and five new-generation photosensitizers that are currently under clinical evaluation were investigated, and all of them produced tumor PDT responses receptive to the interaction with BCG effects. This lack of discrimination may appear remarkable, since there are considerable differences in the mode of tumor damage induction by Photofrin, *m*THPC, BPD, NPe6, Lu-Tex and ZnPc. They vary largely in lipophilic/hydrophilic properties (hence require different delivery vehicles) and pharmacokinetic parameters, which are responsible for their different tumor tissue and subcellular localization patterns (1,2,44,45). Vascular effects induced in target tumor tissue differ greatly among these photosensitizers. On one side are BPD, NPe6 and Lu-Tex, whose effectiveness depends on their high circulating levels at the time of

light treatment, their action is primarily focused on tumor vasculature (6,46,47,48). On the other is ZnPc that does not target vasculature and shows no initial vascular destruction (36). The action of Photofrin and *m*THPC inflicts direct damage to both tumor vasculature and parenchyma (49).

The fact that BCG enhances the antitumor action of all these different photosensitizers suggests that the interaction does not take place during the early phase of tumor ablation but, rather, at a later phase at the level of responses involved in preventing tumor recurrence. This conclusion is supported by the fact that BCG treatment delayed by 1 week after PDT is still effective in enhancing the therapy outcome (Fig. 4 ). In accordance with this, BCG administration was not found to affect significantly the PDT-induced accumulation of host myeloid cells in the treated tumor. On the other hand, the antitumor activity of T lymphocytes, which takes time to develop and lasts over a longer posttherapy time period, appears to be augmented by the adjuvant effect of BCG.

Tumor-draining lymph nodes are the sites of generation of tumor-sensitized lymphocytes and their accumulation. The changes detected after Photofrin-PDT in the populations of lymphocytes residing in these lymph nodes, particularly the increased incidence of $CD44^{high}CD45RB^{low}$ fraction among T cell population (*i.e.* immune memory cells)(Fig. 5 ), are consistent with the induction of tumor immunity. We have shown earlier that the spleens of mice cured of EMT6 tumor by PDT contain cells that can secure the rejection of this specific tumor, which serves as an indirect proof of their immune memory character (12). In this report, these cells were directly demonstrated at markedly elevated levels in the lymph nodes draining PDT-treated tumors. Moreover, it is shown that the adjuvant BCG immunotherapy promotes further increase in their incidence.

Immune memory cells survive in the host for extended time, retaining their capacity to recognize specific antigens and kill the cells bearing these antigens (41). Thus, the therapeutic benefit of adjuvant BCG therapy is based on amplifying the T lymphocyte–mediated immune response against PDT-treated tumors, which is responsible for preventing the recurrence of malignant lesions and ensuring their permanent eradication.

It is important to note that the adjuvant BCG therapy diminished the tumor recurrence rate but was generally ineffective in delaying the time required for the recurrence of PDT-treated EMT6 tumors. Thus, this immune vaccine seems to improve the eradication of small foci of residual cancer cells, while it appears incapable of controlling larger cell numbers of rapidly growing EMT6 sarcoma. Hence, the beneficial effect of adjuvant BCG may turn out to be even greater with PDT-treated slow growing human tumors.

As alluded to in the “Introduction,” the PDT-induced inflammatory response is elicited through a massive and rapid release of proinflammatory mediators liberated from cancer cell membranes, vascular endothelium and tumor stromal elements, as all of them sustain damage from photooxidative lesions. This inflammatory response was suggested to represent a critical initial development that orchestrates events leading to the recognition of antigens of PDT-treated tumors and the ensuing generation of tumor immunity (1,3,5). This prompted us to examine whether the observed beneficial effect on PDT response of bacterial vaccines and other nonspecific immune agents (3,14–17) could be based on the augmentation of inflammatory reaction in PDT-treated tumors. However, as exemplified by the ineffectiveness of treatment with FIA (Fig. 1 ), the amplification of nonspecific inflammatory response limited to the activation of phagocytes and vascular leakage does not promote the destruction of PDT-treated tumors. In contrast to FIA, BCG treatment triggers a more complex release of immunoregulatory cytokines and other mediators, elicits the engagement of both myeloid and lymphoid immune cells and directly stimulates cell-mediated immunity (24,25,28–30).

The overall findings of this study strongly support the idea that combining PDT and BCG immunotherapy greatly enhances the therapeutic outcome reached with each of these modalities used alone. The therapeutic benefit of this combination could be further verified using orthotopic bladder tumor model (50) before it is clinically tested for the treatment of superficial carcinomas of urinary bladder where BCG is already established as a gold standard (18). Repeated intravesical BCG for these lesions is not always effective in preventing their recurrence and is sometimes associated with undesired side effects (51). Using PDT in conjunction with BCG treatment may result in improved cure rates and reduce the incidence of bladder tissue

damage by using protocols with less BCG instillations and decreased doses of photosensitizer–light combinations for PDT. As these two modalities show beneficial interaction even with delayed BCG treatment, this can be exploited for limiting the adverse inflammatory damage of bladder tissue that may arise with simultaneous application of PDT and BCG.

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FIGURES Return to TOC

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Figure 1.  The effect of FIA on the response of EMT6 tumors to PDT. Mice bearing subcutaneously growing EMT6 tumors were administered the photosensitizer *m*THPC (0.2 mg/kg, intravenously [i.v.]) and 24 h later the tumors were exposed to the light dose of 30 J/cm^2. One group of mice was injected with FIA (0.1 mL s.t.) immediately after light treatment. The mice were observed afterwards for signs of tumor growth. Tumor-free mice at 90 days after PDT were considered cured. The difference in tumor response between the two treatment groups is not statistically significant

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Figure 2.  The response of EMT6 tumors to the treatment with PDT, BCG or their combination. The BCG treatment (1×10^7 CFU per mouse s.t.) was performed either alone or in conjunction with PDT. In the latter case, BCG was injected either 24 h before or immediately after photodynamic light exposure. The PDT treatment was performed as described in Fig. 1  , except that the light dose was lowered to 20 J/cm^2. The response of tumors was evaluated as described in Fig. 1  , while BCG-alone treatment was assessed based on tumor volume measurement. The statistical significance for the difference in tumor response compared to PDT only: $^{**}P < 0.01$; $^{***}P < 0.001$. The differences in tumor volume between BCG and control saline groups were statistically significant only 7 days after treatment ($^{*}P < 0.05$)

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Figure 3.  The effect of BCG on the response of EMT6 tumors to PDT mediated by photosensitizers BPD, NPe6, Lu-Tex and ZnPc. The photosensitizer dose (i.v.), interval to light treatment and light dose, respectively, were 2.5 mg/kg, 3 h, 60 J/cm^2 for BPD (upper left graph), 2.5 mg/kg, 4 h, 120 J/cm^2 for NPe6 (upper right graph), 11.65 mg/kg, 3 h, 100 J/cm^2 for Lu-Tex (lower left graph) and 0.5 mg/kg, 48 h, 120 J/cm^2 for ZnPc (lower right graph). The BCG treatment, performed immediately after PDT light exposure, was as described in Fig. 2  , except that one group of mice treated with BPD-mediated PDT received intraperitoneal BCG instead of subtumoral. The response of tumors was evaluated as described in Fig. 1  . The statistical significance for the difference in tumor response compared to PDT only: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

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Figure 4.  The impact of delaying the after PDT treatment with BCG. The mice bearing EMT6 tumors were administered Photofrin (10 mg/kg, i.v.) and 24 h later the tumors exposed to 70 J/cm^2. The tumor-localized BCG treatment (1×10^7 CFU per mouse) was performed 7 days after PDT light. The response of tumors was evaluated as described in Fig. 1  . The statistical significance for the difference in tumor response compared to PDT only: $^{*}P < 0.05$

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Figure 5.  The incidence of memory T lymphocytes in lymph nodes draining EMT6 tumors treated by PDT, BCG or their combination. The tumors were treated either by Photofrin-based PDT (Photofrin 10 mg/kg i.v. followed 24 h later by tumor light exposure of 100 J/cm^2), BCG (1×10^7 CFU per mouse, s.t.) or PDT followed immediately by BCG. The mice were sacrificed and inguinal lymph nodes excised 6 days after treatment (13 days after tumor implant). Flow cytometry analysis of cells released from the lymph nodes and stained with various antibodies was used to identify memory T lymphocytes ($CD3^{high}CD44^{high}CD45RB^{low}$) and determine the incidence of major immune cell types (pie insets). Statistically significant difference compared to the values in control tumors: $^{**}P < 0.01$: $^{***}P < 0.005$

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†*Abbreviations:* BCG, Bacillus Calmette–Guérin; BPD, benzoporphyrin derivative; CFU, colony-forming units; FIA, Freund's incomplete adjuvant; i.v., intravenously; Lu-Tex, lutetium texaphyrin; *m*THPC, tetra(*m*-hydroxyphenyl)chlorin; NPe6, mono-L-aspartyl-chlorin *e*6; PDT, photodynamic therapy; s.t., subtumorally; TFA, trifluoroacetic acid; ZnPc, zinc phthalocyanine.

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