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Immune response after photodynamic therapy increases anti-cancer and anti-bacterial effects

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Abstract

Photodynamic therapy (PDT) is a clinically approved procedure for treatment of cancer and infections. PDT involves systemic or topical administration of a photosensitizer (PS), followed by irradiation of the diseased area with light of a wavelength corresponding to an absorbance band of the PS. In the presence of oxygen, a photochemical reaction is initiated, leading to the generation of reactive oxygen species and cell death. Besides causing direct cytotoxic effects on illuminated tumor cells, PDT is known to cause damage to the tumor vasculature and induce the release of pro-inflammatory molecules. Pre-clinical and clinical studies have demonstrated that PDT is capable of affecting both the innate and adaptive arms of the immune system. Immune stimulatory properties of PDT may increase its beneficial effects giving the therapy wider potential to become more extensively used in clinical practice. Be sides stimulating tumor-specific cytotoxic T-cells capable to destroy distant untreated tumor cells, PDT leads to development of anti-tumor memory immunity that can potentially prevent the recurrence of cancer. The immunological effects of PDT make the therapy more effective also when used for treatment of bacterial infections, due to an augmented infiltration of neutrophils into the infected regions that seems to potentiate the outcome of the treatment.

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Keywords

Photodynamic therapy; Anti-tumor immunity; T-cell activation; Damage-associated molecular patterns; Inflammatory cells

INTRODUCTION

The ideal therapy for cancer should be able to selectively destroy the tumor cells at the primary site and at the same time trigger the immune system to recognize any remaining or recurring cancer cells. Compared to other unspecific and/or immunosuppressive cancer therapies such as chemotherapy, ionizing radiation and surgery, photodynamic therapy (PDT) might have these desirable properties. PDT is a procedure that consists of three components: A photosensitizer (PS), light of appropriate wavelength to excite the PS and molecular oxygen[1,2].

None of these three components is individually toxic, but when combined together they initiate a photochemical reaction that culminates in the generation of highly reactive oxygen species (ROS)[3]. Most of the PSs used in PDT are based on a tetrapyrrole structure, similar to that of the protoporphyrin contained in hemoglobin[4]. They have an absorption peak between 600 and 800 nm (red to deep red), since light at lower wavelengths would not penetrate efficiently through the tissue and light at longer wavelengths than 800 nm would not have sufficient energy to initiate a photochemical reaction and generate a substantial yield of ROS[4].

The ROS produced during PDT can directly kill tumor cells by induction of necrosis and/or apoptosis[5] and damage the tumor vasculature, leading to depletion of oxygen and nutrients in the tumor[6,7]. As a result of this traumatic insult to the tumor and its microenvironment, a strong acute inflammatory reaction is provoked at the targeted site[1]. The acute inflammatory response following PDT causes infiltration of host innate immune cells that carry out the removal of damaged cells. Acute inflammation also seems to be implicated in the development of adaptive anti-tumor immunity[1]. In particular, the efficacy of PDT in some models has been shown to be dependent upon such induction of anti-tumor immunity. Early studies showed that while PDT of EMT6 tumors exhibited curative effects and long-term tumor control in Balb/c mice, the long-term protection from tumors was lost when PDT was performed in either *scid* (which lack T and B cells), or nude (which lack T cells) immune-compromised mice. However, when the *scid* mice were reconstituted with splenic T cells or bone marrow cells from Balb/c mice, the curative effect of PDT was restored[8,9].

While the immune stimulatory effects of PDT have been widely studied, although not completely understood in cancer models, much effort still has to be done to understand these effects of PDT in microbial infections. Tanaka *et al*[10] discovered that the therapeutic effect of PDT in a mouse model of bacterial arthritis was dependent on the attraction and accumulation of neutrophils into the infected region and could also produce a protective effect if carried out before infection. This review will focus on the current knowledge of the beneficial immunological effects of PDT for cancer and bacterial infections. A list of

notable publications that show that PDT can activate different constituents of the immune system is provided in Table 1.

DAMAGE-ASSOCIATED MOLECULAR PATTERNS

After the traumatic insult to the tumor induced by PDT, one of the first events occurring at the treatment site is the generation of "danger" signals, so called damage-associated molecular patterns (DAMPs) or cell death-associated molecular patterns (CDAMPs) that serve as warning signals in innate immunity[11-14]. DAMPs play a similar role to that of pathogen-associated molecular patterns, but instead of being associated with pathogenic microbes, they are associated with host tissue damage. DAMPs are endogenous intracellular molecules normally "hidden" within living cells, but upon exposure or secretion from dying and/or damaged cells, they acquire immune-stimulatory properties. DAMPs are thought to be the key mediators of the immunogenicity of tumor cells killed by PDT *via* necrosis or apoptosis. They constitute alarm signals warning that "self-altered" antigens were released from dying cells; the immune system recognizes them and triggers a vigorous immunological response. It is generally accepted that while necrotic cells are pro-inflammatory and immunogenic, some forms of apoptotic cells are efficiently engulfed and disposed of by macrophages and other phagocytic cells, therefore they should not induce inflammation and are unlikely to stimulate the immune system[15,16]. However, it has been reported that under certain circumstances, other forms of apoptotic cells such as tumor cells undergoing apoptosis by some particular cancer therapies can effectively generate an immune response[17,18]. In this case the process is defined as "immunogenic apoptosis" *vs* the conventional "nonimmunogenic apoptosis"[16,19,20].

It is conceivable that while the physiological programmed cell death is non-inflammatory and non-immunogenic, some cancer therapies (such as particular forms of chemotherapy and PDT) cause tumor damage, and produce an immunogenic form of apoptosis characterized by release of DAMPs and enhancement of inflammation.

The release of DAMPs after PDT has been investigated in some studies[11,12,21]. Korbelik *et al*[22] found that squamous cell carcinoma VII (SCCVII) cancer cells treated by *in vitro* photofrin-PDT expose on the surface heat shock proteins (HSPs) such as HSP60, HSP70 and glucose-regulated protein 94 (GRP94) and release HSP70 to the extracellular space. Interestingly, when PDT was applied in *in vivo* settings, they found a different spectrum of DAMPs exposed on the surface of treated SCCVII cells. While HSP70 was still exposed, HSP60 and GRP94 were no longer detected and replaced by GRP78 on the surface of PDT-treated SCCVII cancer cells. This indicated for the first time that the DAMPs associated with PDT can differ in the same cancer cells between *in vitro* and *in vitro* settings[22].

It is worth mentioning also that the spectra of DAMPs exposed and/or released after PDT correlate with the sub-cellular localization patterns of the PS, where the ROS-based stress is originated. For instance, PSs targeting the endoplasmatic reticulum (*e.g.*, hypericin) are known to cause surface exposure of calreticulin (CRT); conversely, Photofrin (whose localization is mostly associated with lipid membranes)-PDT, has been linked primarily to surface exposure of HSP70[23,24].

Further investigations on cellular and molecular mechanisms are certainly required to establish in more detail the correlations between DAMPs and PDT. However, the most important examples of DAMPs which are produced after PDT reported so far are HSPs, CRT, adenosine triphosphate and other mediators[21,22,25]. Table 2 lists the DAMPs that have been reported to be produced after PDT.

INFLAMMATION AND INNATE IMMUNE RESPONSES IN ANTI-CANCER PDT

The PDT-induced oxidative stress and traumatic insult to the tumor microenvironment are known to stimulate the release or expression of various proinflammatory mediators [tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1, complement proteins, HSPs and arachidonic acid metabolites] from the treated site[26]. Moreover, as men tioned above, immunogenic DAMPs are released after PDT and they can be detected by the innate immune cells that are programmed to detect microbial invasion[27]. For these reasons, innate immune cells such as monocytes or macrophages, neutrophils and dendritic cells (DCs) are recruited to the treated site and infiltrate in large numbers to attack what is expected to be a microbial invasion but turns out to be damaged tumor cells[28]. The primary function of the inflammatory cells is to neutralize the DAMPs by engulfing and eliminating the cellular debris as well as compromised tissue components. This promotes local healing with restoration of normal tissue function. At the onset of PDT-induced inflammation, the tumor vasculature undergoes significant changes and become permeable for blood proteins and pro-adhesive for inflammatory cells *via* over-expression of adhesion molecules (Intracellular Adhesion Molecule 1, Vascular Cell Adhesion Molecule 1, selectins)[27], thus favoring the massive infiltration of the immune cells into the tumor.

The inflammatory cells are known to be necessary to achieve efficacious PDT, as several studies have shown that their depletion (or inhibition of their activity) diminishes the therapeutic effect of the treatment[9,29,30]. Among all the cytokines involved in the PDT-induced inflamma-tory process, IL-1β and IL-6 seem to play the most important role[26,31] and conversely, IL-10 and transforming growth factor (TGF)-β seem to hamper PDT-effects as their blockade remarkably improves the cure rates after PDT[27]. Also, blocking the function of various adhesion molecules can affect the efficacy of PDT[26,32]. Figure 1 shows the important cells and mediators that are activated in the tumor environment after PDT of a tumor.

Although PDT is a local treatment, its effect is not limited to the local site, but it can induce a potent acute phase response with systemic consequences[33]. Studies in mouse models have shown that PDT leads to drastic rise in serum levels of acute phase reactants such as serum amyloid P components (SAP), C-reactive protein (CRP) and mannose-binding lectin A (MBL-A)[34]. SAP and CRP belong to the pentaxin family proteins and are involved in acute immunological responses[35]. They are specialized in facilitating the phagocytosis and removal of dying cells such as those killed in PDT-treated tumors. SAP production and release is a hallmark acute phase reactant response in mice, but in humans CRP is a more important acute phase reactant than SAP and PDT dose-dependent up-regulation of CRP has been demonstrated in human lung tumor A549 cells[35]. MBL-A is another important acute phase reactant with functional attributes similar to SAP[36].

Furthermore, a rapid increase in peripheral neutrophils is observed immediately after PDT and it is still present 24 h later, that is correlated with the influx of neutrophils into the treated tumors[37].

ADAPTIVE IMMUNE RESPONSES IN ANTI-CANCER PDT

The PDT-induced local and systemic inflammatory responses can enhance the development of an adaptive immune response capable of protecting the host organism in an antigen-specific manner, owing to immunological memory. It can be asked what is mediating the crosstalk between the innate and adaptive arms of the immune system after PDT. It has been realized that PDT enhancement of adaptive anti-tumor immunity involves the activation of DCs. DC are stimulated by the recognition of DAMPs/CDAMPs released and/or exposed by dying tumor cells[38]. One of the best characterized DAMPs induced by PDT is HSP70, which is released after PDT and forms stable chaperone complexes with cytoplasmic tumor antigens. Thereafter, the HSP-antigen complexes bind to the danger signal receptors, Toll-like receptors 2 and 4[39] on the surface of DCs, which are most potent antigen presenting cells (APCs). In the absence of inflammation DCs remain in an immature state, but when tissue inflammation and release of DAMPs occur, they mature and migrate in large numbers to the draining lymph nodes. The transition to the mature state of DC involves the upregulation of surface major histocompatibility class I and II molecules (MHC I and MHC II) and of the costimulatory molecules CD80 and CD86. These changes allow the DCs to express peptide-MHC complexes at the cell surface and prime efficiently $CD4^+$ T helper cells and $CD8^+$ cytotoxic T lymphocytes (CTLs) and hence to initiate an adaptive immune response. Figure 2 shows the process by which DCs engulf tumor antigens, become activated, traffic to lymph nodes where antigen specific T-cells proliferate and then return to attack remaining tumor cells.

The generation of $CD8^+$ effector and memory T cell induction is generally, but not always dependent on $CD4^+$ helper T cells[40-42]. Kabingu *et al*[43] showed in fact that $CD8^+$ T cell-mediated immunity is independent of $CD4^+$ T cells and depends instead on natural killer cells. Also other studies suggest that $CD8^+$ cells play the most critical role in PDT mediated anti-tumor immunity, as in the absence of their activation and/or tumor infiltration the efficacy of PDT is reduced[9,43]. Furthermore, it has been shown that adoptive transfer of bare $CD8^+$ T cells to immunocompromised *scid* mice can significantly restore PDT efficacy[8].

The adaptive immunity is not provided only by antigen-specific T cells, but also by B cells. B cells produce antigen-specific immunoglobulins, mounting the so called humoral immune response. So far there is only one study showing that the activation of humoral immunity is implicated in the PDT-induced systemic antitumor protection, as seen by (1) increased serum IgG titers after PDT; (2) production of antibodies against existing antigens; and (3) marked B-cell infiltration in the tumor rim 24 h after PDT[44]. Nonetheless, the importance of the humoral components to the tumor eradication process remains unclear and needs further investigations.

ROLE OF TUMOR ANTIGENS IN THE ANTI-TUMOR IMMUNE RESPONSE

Tumor antigens (TAs) represent a sort of “bait” for the immune system, since they activate DCs and allow the antigen-specific CTLs to recognize and destroy the tumor cells. Some TAs have been well defined in murine and human tumors[45] and are generally classified in three distinct groups: (1) Antigens encoded by cancer-testis genes expressed in various tumors, but not in normal tissues, such as the mouse gene *P1A* and human genes of the melanoma antigen (MAGE)-type, B MAGE and G antigen families[46-51]; (2) Differentiation antigens of the melanocytic lineage, which are present on most melanomas but also on normal melanocytes (*i.e.*, melanoma antigen recognized by T-cells 1, gp100)[51-53]; and (3) Antigens that result from tumor-specific mutations in genes which are expressed in all tissues (*i.e.*, p53, p16) or come from viruses (*i.e.*, Epstein-Barr virus, Hepatitis B virus)[54-58]. Successful immunotherapeutic strategies targeting the TAs have been developed in preclinical studies and early-phase clinical trials[59,60], and our group was the first to realize the importance of TAs expression in PDT anti-tumor immunity.

We showed that a vascular PDT regimen was able to produce 100% of long term cures and rejection of re-challenge when tumors were induced in C3H mice with green fluorescent protein-expressing radiation-induced fibrosarcoma cells, but not with their wild-type counterpart[61]. The same effect was observed when we used a pair of equally lethal Balb/c colon adenocarcinomas: The antigen negative CT26 wild-type and the CT26.CL25 transduced with *lacZ* gene, and thus expressing the tumor antigen β-galactosidase[62]. We could further show that PDT of antigen positive tumors, but not of antigen neg ative tumors could trigger a highly potent antigen-specific systemic immune response capable to induce regression of distant untreated tumors. Recently we employed the P1A antigen positive mouse mastocytoma P815 wild-type and P1A antigen negative P1.204 (P815 derived) tumor models to study the antigen-specific PDT-induced antitumor immunity[63]. This model is clinically more relevant than others as the P1A is a naturally occurring murine cancer antigen, homologue of the human MAGE-type antigen[64]. We found that tumor cures, significantly higher survival and rejection of tumor rechallenge were obtained with P815, but not with P1.204 tumors that lack the antigen.

The role of the TAs in PDT anti-tumor immunity has been recently investigated also in the clinical setting. In a study published by Kabingu *et al*[65] in 2009, they demonstrated for the first time the enhancement of systemic immune reactivity to a basal cell carcinoma (BCC) associated TA (Hedgehog-interacting protein 1) following PDT in patients. These novel findings in patients are important as they are supporting the results in preclinical models, but more effort needs be done in clinical trials to elucidate the PDT-induced systemic immune responses to tumor antigen.

IMPACT OF T REGULATORY CELLS IN THE ANTI-TUMOR IMMUNE RESPONSE

In addition to directly stimulating anti-tumor immunity by triggering DCs and T cells activation, PDT may also interfere with immune-suppressive T cells. The main class of T cells suppressing the immune response consists of $CD4^+CD25^+FoxP3^+$ T regulatory cells

(Treg)[66]. The involvement of Treg in both autoimmune disease[67] and cancer[68] has been extensively described in mice and humans. Treg are thought to mediate their immunosuppressive effects by multiple mechanisms[69]. Treg express the protein receptor cytotoxic T-lymphocyte antigen 4 (CTLA-4), which is similar to the T-cell costimulator protein CD28. CTLA-4 binds with much higher affinity to B7-1 and B7-2 costimulatory molecules on APCs compared to the equivalent molecule CD28 and transmits inhibitory signals, rather than stimulatory[70].

Treg are generally classified into two main subpopulations: Natural Treg and induced Treg[71]; the former are found in the thymus and thought to have T-cell receptors that recognize self-antigens, therefore important in the prevention of autoimmune disease, the latter can be induced and differentiate in the periphery, *i.e.*, upon influence by TGF-β in the tumor microenvironment[72]. Several studies have shown that Treg inhibit the generation of immune responses against tumors[71], but on the other hand, their depletion *in vivo* facilitates tumor eradication and enhances-anti-tumor immunity[73-75]. A summary of the features of Treg is provided in Table 3.

Our research group was the first to investigate the potential relationship between PDT and Treg and we realized that Treg play an important and negative role in PDT anti-tumor immunity. We observed that if Treg are depleted by low-dose cyclophosphamide (CY) (a traditional cytotoxic cancer drug that at low doses selectively depletes Treg[76]) prior to PDT, the anti-tumor immune responses are potentiated and a memory immunity is generated against metastatic J774 tumors[77]. This effect was not seen when PDT and CY treatments were given separately or when PDT was combined with high-dose CY that destroyed all T-cells not just Treg. Another recently completed study involving the colon adenocarcinoma CT26 wild-type tumor model revealed that the combination of PDT with low-dose CY produced a dramatic improvement in long-term survival, compared with either treatment alone and led the development of immune response to the mouse cancer shared/auto-antigen gp70[78]. Moreover, this combination treatment activated a long-lasting immune memory, that could however be uncovered only when Treg were depleted again by CY before rechallenge. These new findings are important, because they emphasizes that one of the most effective approaches for optimally improving anti-cancer PDT would be by restraining host's regulatory immune cell populations.

CLINICAL EVIDENCE FOR THE IMPACT OF THE IMMUNE SYSTEM IN ANTI-CANCER PDT EFFECTS

The first clinical use of PDT for cancer in modern times dates back to the beginning of the 20th century when Von Tappeiner *et al*[79] used eosin as topical PS combined to sunlight to treat facial BCC. That first trial was successful as out of 6 patients, 4 showed complete tumor resolution. Many years later, in the 1970s, hematoporphyrin derivative (HPD) and light were administered to the tumor area of patients with bladder cancer[80] and resulted in positive outcomes. In the same decade Dougherty *et al*[81] tested for the first time HPD-PDT in a large series of patients with skin tumors reporting striking results: Complete or partial responses were observed in 111 out of 113 patients.

Since then, over 200 clinical trials for PDT as treatment for a large variety of tumors have been carried out. Some clinical studies have demonstrated that PDT efficacy seems to depend on antitumor immunity also in patients. Dragieva *et al*[82] published a study comparing the efficacy of PDT for actinic keratosis and Bowen's disease in immune-competent patients *vs* immune-suppressed transplant patients. The two groups of patients showed comparable initial response, however the immune-suppressed patients had an increased propensity to develop new lesions after the treatment. It has also been shown that patients with vulval intraepithelial neoplasia (VIN) expressing MHC I molecules on the tumor cells were more likely to respond to aminolevulinic acid-PDT compared to patients whose tumors had down-regulated MHC I molecules[83]. MHC I recognition is critical for activation of $CD8^+$ T cells and the down-regulation of MHC I molecules is one of the mecha-nisms used by tumors to evade immune recognition in general and PDT-induced immunity in particular. VIN patients who did not respond to PDT had significantly lower $CD8^+$ T cell infiltration into the treated tumors compared with responders, confirming the important role of $CD8^+$ CTLs in PDT efficacy. The first clinical case of systemic PDT-immune response observed in patients has been published in 2007: PDT of multifocal angiosarcoma of the head and neck located on the right upper limb of a patient, resulted in a spontaneous regression of the untreated distant tumors on the contralateral left upper limb, accompanied by increased immune cell infiltration[84]. Two years later Kabingu *et al*[65] found that PDT treatment of BCC lesions enhanced the reactivity of patients lymphocytes against Hip1, a known BCC-associated TA, as seen by increased secretion of IFN-γ by patients lymphocytes following incubation with the TA derived peptide.

PDT FOR INFECTIONS

Although PDT was discovered in the field of microbiology over 100 years ago[85], up to now PDT has been studied and applied mainly as anticancer treatment. The discovery of antibiotics in 1940s revolutionized the treatment of infectious disease, limiting the development of other potential alternative anti-microbial treatments like PDT. However, the recent worldwide increase of resistance to antibiotics has strongly enhanced the interest in alternative therapeutic strategies for the treatment of infections. PDT is capable of killing a large variety of pathogens such as bacteria, parasitic protozoa, fungi, yeasts and viruses. Furthermore, PDT does not induce resistance itself and it is a non-invasive method. PDT is more effective in inactivating Gram (+) bacteria compared to Gram (−) due to the different structure of the cell walls[86]. The membrane of Gram (+) bacteria is surrounded by a permeable layer of peptidoglycan and lipoteichoic acid that allows the PS to pass through it[87]. Gram (−) species have an inner cytoplasmic membrane and an outer membrane, which are separated by a peptidoglycan-containing periplasm. The outer membrane constitutes a permeability barrier between the cell and its environment, limiting the PS penetration. Fungal cell walls have a moderately thick layer of chitin and β-glucan that result in a barrier with moderate permeability. Several *in vitro* and *in vivo* studies have been carried out to verify the efficacy of PDT for viral infections, soft tissues infections, oral and dental infections produced by different strains of bacteria. PDT has been shown to work efficiently against *Escherichia coli* and *Pseudomonas aeruginosa* in excisional wounds[88,89] and against *Acinetobacter baumannii* and *Staphylococcus aureus* in burn infections[90,91].

There are reports of PDT on its effects on certain species of fungus, including both filamentous fungi (*Tricophyton*[92] and *Aspergillus*[93]) and yeasts (*Saccharomyces*[94] and *Candida albicans*[95,96]). Also several types of virus have been tested for the affection by PDT, including herpes viruses HSV-1[97] (PDT by methylene blue and light), enveloped RNA viruses from two different families, Semliki Forest Virus (*Togaviridae*) and vesicular stomatitis virus (*Rhabdoviridae*) (PDT by buckminsterfullerene and light)[98] and others[99].

Some clinical trials for PDT have been carried out for dental, gastric and dermatological infections such as acne as well as rosacea, a condition in which microbes may play a role in the pathogenesis[100].

IMMUNE RESPONSES IN ANTIBACTERIAL PDT

While the immune stimulating effects of PDT have been widely studied in cancer models, little is known about the immunological effects of PDT in bacterial infections. A recent study published by Tanaka *et al*[10] convincingly demonstrated for the first time that *in vivo* PDT can stimulate an innate immune response. They used a mouse model of bacterial arthritis (*Staphylococcus aureus* infection in the knee joint) and observed a strong infiltration of neutrophils in the PDT-treated area. In order to investigate the role of neutrophils in the PDT-mediated bacteria inactivation, they administered anti-GR-1 (anti-neutrophil) antibody as well as antibodies to several pro-inflammatory mediators. The administration of such antibodies resulted in loss of the therapeutic effect of PDT. This suggests that not only killing of bacteria, but also attraction and accumulation of neutrophils into the infected regions were required mechanisms to achieve PDT-mediated clearance of bacterial infections. Additionally, PDT was tested also as a preventive therapeutic approach and delivered prior to the bacterial inoculation into the knee. PDT-mediated infiltration of neutrophils prevented the subsequent inoculation of bacteria from establishing the infection and again, such an effect was abrogated when antibodies against GR-1 and proinflammatory mediators were administered. To the best of our knowledge, this is the first demonstration of a protective innate immune response against a microbial pathogen being induced by PDT. It is well known that bacterial phagocytosis by innate immune cells such as neutrophils, plays a crucial role in the elimination of invading bacteria and, therefore, malfunction of the phagocytic immune system renders the host more susceptible to bacterial infections. Hence, it would be desirable to apply an antimicrobial PDT regimen that causes direct photoinactivation of bacteria, but at the same time that can minimize the damage to the host's neutrophils.

As described above, evidence indicated that PDT of cancer triggers the activation of both innate and adaptive arms of the immune system, while the early results from the bacterial infection models suggest that PDT is capable of stimulating (at least) the innate immune system. The biggest difference, however, could be in the stimulation of T- and B-cell-mediated adaptive immune responses. As antibodies produced by B cells are generally the most effective component of the immune response against bacterial infection, B cells are expected to be the main actors in the post-PDT immune response towards bacteria. However, to the best of our knowledge, nothing is known yet about humoral responses induced by PDT against bacterial infection.

On the other side, while the involvement of B cells in PDT-induced antitumor immunity still needs more investigation, it is widely accepted that the activation of T cell responses play a pivotal role in PDT-mediated immunity towards treated tumors.

CONCLUSION

Several studies in pre-clinical and clinical settings have demonstrated that PDT is capable of pronouncedly activating both the innate and adaptive arms of the immune system. Such effects on the immune system appear to be PDT regimen dependent and strictly linked to the degree of inflammation induced by PDT.

It has been speculated that PDT regimens causing a high degree of acute inflammation are better at immune activation compared to those in which the acute inflammation is lower. However, increase in inflammatory mediators could promote tumor cell growth in certain circumstances[101]. Moreover, PDT has been linked also to immunosuppressive effects. Such immunosuppressive effects have been established in model of suppression of induction of contact hypersensitivity (*i.e.*, afferent immune response), which involves the application of a hapten to the skin, followed by re-challenge[102], and suppression of delayed-type hypersensitivity (Mantoux) reactions (*i.e.*, efferent immune response) for instance in healthy Mantoux-positive volunteers[103,104]. In particular, such immunosuppressive responses seem to be dependent on the rate of light delivery[105] and anatomic site of PDT[106].

Further studies using a better targeted and dose-controlled PDT treatment would help to expand the knowledge on the activation/suppression of the immune system and the possibilities to improve it in clinical practice.

The proven ability of PDT to trigger inflammation and improve the anti-tumor immune response could be successfully employed in tandem with other treatment modalities, to combat cancer and to achieve long-term tumor control. Nevertheless, up to now PDT remains clinically underutilized. We must realize that with all probability it will take several years of further investigations and clinical trials before the use of PDT becomes a clinically accepted standard practice in cancer patients.

The innate immune responses seem to be of crucial importance also in the relatively new field of PDT as anti-microbial treatment. The activation of neutrophils after PDT, their mobilization from the bone marrow and their attraction to the site of inflammation appear to be important mechanisms, significantly potentiating the antibacterial effects, *e.g.*, in bacterial arthritis mouse models. However, it still remains to be elucidated whether the activation of the host neutrophils is applicable also to other infection models, with other classes of pathogens and/or using different PS. Many years of intense research will be required providing answers to these intriguing questions.

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Core tip

The immune stimulatory properties of photo-dynamic therapy (PDT) make this therapy one of the most promising therapeutic procedures for the management of cancer lesions and microbial infections. This review will focus on the current knowledge of the innate and adaptive immune responses induced by PDT against tumors and pathogens.

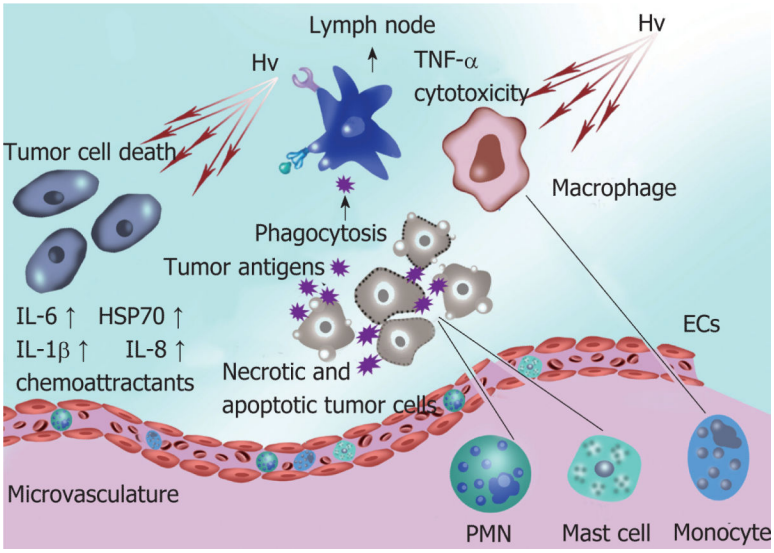

Figure 1. Innate immune responses in anti-cancer photodynamic therapy

Photodynamic therapy of tumors leads to the development of local inflammation mediated by the release of danger signals and cytokines. Various cells of the immune system infiltrate into the treated area. ECs: Endothelial cells; HSP70: Heat-shock protein; Hv: Light; PMNs: Polymorphonuclear neutrophils; TNF: Tumor necrosis factor; IL-6: Interleukin-6. Original figure based upon Ref. [115] and Ref. [116].

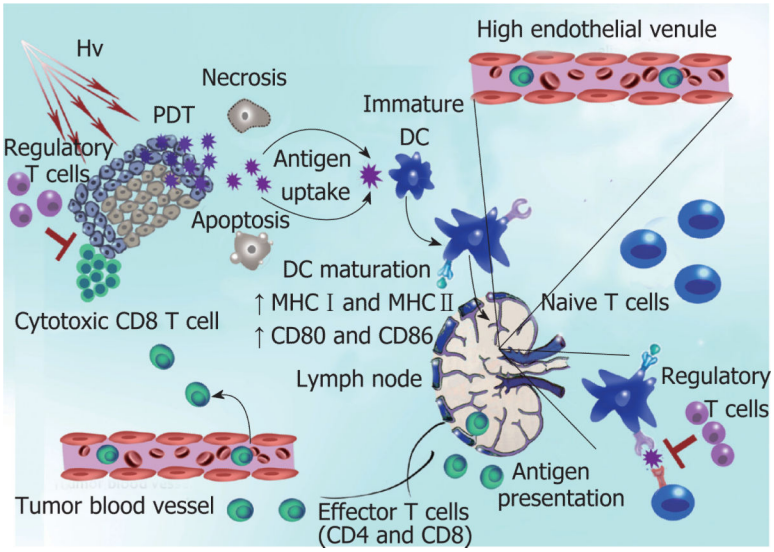

Figure 2. Stimulation of adaptive anti-tumor immunity by photodynamic therapy

PDT-treated tumor cells release the antigens, which are phagocytosed by DCs and presented to naive T cells in the tumor draining lymph node. Activated effector T cells return in circulation and migrate to the tumor. Regulatory T cells seem to inhibit the immune responses after PDT. DCs: Dendritic cells; Hv: Light; MHC I : Major histocompatibility class I ; PDT: Photodynamic therapy. Original figure based upon Ref. [115] and Ref. [116].

Table 1

Milestone studies on effects of photodynamic therapy affecting the immune system

Immune components	Immunomodulatory effect of PDT	Ref.
Pro-inflammatory cytokines	Production of pro-inflammatory cytokines after PDT *in vivo*	[26]
Macrophages	First evidence of cytokine production by PDT-treated macrophages *in vivo*	[107]
Dendritic cells	DCs can efficiently phagocytose PDT-treated tumor cells in *in vivo* experiments. Immature DCs administered in combination with PDT produce effective antitumor response *in vivo*	[38,108]
NKs	Role of NKs in immune response after PDT, control of distant untreated tumors	[43]
Neutrophils	Evidences that neutrophils have a crucial role in the PDT response *in vivo*	[30,109]
Memory immunity	First demonstration that a specific antitumor memory immunity is induced after PDT: resistance to tumor rechallenge in animals cured by PDT	[110]
T lymphocytes, memory immunity	Essential role of host T lymphocytes in immune response after PDT: curative effect of PDT in immune-competent Balb/c mice, but not in immune-suppressed *scid* mice. Adoptive transfer of splenocytes from PDT-cured mice to *scid* mice confers resistance to tumor rechallenge	[8,111]
Treg	Evidences for the role of Treg in inhibiting the immune response after PDT	[77]
Patient lymphocytes	First demonstration that an antigen-specific immune response can be observed after PDT	[65]

PDT: Photodynamic therapy; DCs: Dendritic cells; NKs: Natural killer cells; Treg: T regulatory cells.

Table 2

Damage-associated molecular pattern molecules that may be released or exposed on the outer leaflet of dying tumor cells after photodynamic therapy

DAMP	Function	Ref.
HSP60, HSP70, HSP90, gp96, GRP94, GRP78	Molecular chaperones that normally reside in intracellular regions/organelles, but under stress they are exposed on the damaged cell surface and prime immunomodulatory processes	[11,21,22,112]
Calreticulin	Calcium binding protein located in intracellular regions/organelles (mostly in ER), but under stress its presence on the PM is augmented. On the PM it acts as “danger signal” and increases the immunogenicity of the dying cells	[11,112]
ATP	High-energy molecule, normally intracellular, but can be released by necrotic and apoptotic cells under particular stresses. Extracellular ATP has the ability to help in chemoattraction of immune cells	[12,112]
Phosphatidylserine	When cells are damaged/dying, phosphatidylserine is transposed from the inner to the outer leaflet and acts as an “eat me” signal by interacting with multiple immune cells receptors, mediating efficient phagocytosis and anti-inflammatory responses	[112,113]
High mobility group box-1	Nuclear chromatin-binding protein; it has prominent cytokine-line properties and when released by dying cells tends to stimulate immune cells to produce various pro-inflammatory cytokines	[11,112]
Calgranulin family members (S100A8, S100A9, S100A12)	Calcium-binding proteins; when released by necrotic cells they act as “find me” signals attracting various immune cells and interacting with immune cell receptor (TLR4/RAGE) to induce the secretion of pro-inflammatory cytokines	[11,112,114]
Cross-linked dimer of ribosomal protein S19	Constituent of small ribosomal subunit; when released by necrotic cells it acts as a chemotactic factor for attracting various immune cells	[11,112]

DAMPs: Damage-associated molecular patterns; ER: Endoplasmatic reticulum; PM: Plasma membrane; HSP: Heat shock protein; GRP: Glucose-regulated protein; ATP: Adenosine triphosphate; TLR4: Toll-like receptor 4; RAGE: Receptor for advanced glycation end-products.

Table 3

Common features of T regulatory cells

Features of Treg	Ref.
Phenotypic and functional specialization	[66-68]
Treg are CD4 CD25 FoxP3 immunosuppressive T cells. They are important for the maintenance of the immune homeostasis and involved in both autoimmune disease and cancer	[71,72]
Cells subpopulations	
Treg are generally classified into nTreg and iTreg. The former are found in the thymus and thought to have T-cell receptors that recognizes self-antigens, therefore important in the prevention of autoimmune disease, the latter can be induced and differentiate in the periphery, *i.e.,* upon influence by TGF-β in the tumor microenvironment	[69,70]
Immunosuppressive mechanisms	
Treg are thought to mediate their immunosuppressive effects by multiple mechanisms, among which Secretion of immunosuppressive cytokines	
High affinity binding of his CTLA-4 receptor to B7-1 and B7-2 costimulatory molecules on antigen presenting cells and transmission of inhibitory signals	
Role of Treg in anti-tumor immunity	[71,73-75]
Treg are known to inhibit the generation of immune responses against tumors. Treg depletion *in vivo* facilitates tumor eradication and enhances-anti-tumor immunity	

nTreg: Natural Treg; iTreg: Induced Treg; CTLA-4: Cytotoxic T-lymphocyte antigen 4; Treg: T regulatory cells; TGF: Transforming growth factor.