

PDT-Associated Host Response and its Role in the Therapy Outcome

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Background and Objectives: The outcome of the treatment of solid tumors by photodynamic therapy (PDT) is critically dependent on the contribution from the host. This host response is provoked by the rapidly induced massive tumor tissue injury delivered by PDT that is experienced as a local trauma threatening the integrity and homeostasis at the affected site.

Study Design/Materials and Methods: Mouse tumor models were extensively employed in pre-clinical studies investigating various aspects of host-tumor interaction following PDT, but important input was also derived from clinical data.

Results: The recognition of this PDT-inflicted insult by innate immune sensors detecting danger signals from the distressed/alterd tumor tissue, triggers host-protecting responses dominantly manifested as acute inflammation that are elicited and orchestrated by the innate immune system. To secure the affected PDT-targeted site, the inflammatory reaction attacks tumor vasculature and then neutralizes the focal source of danger signals by eliminating the injured tumor cells.

Conclusion: The provoked highly intensified phagocytosis of dead tumor cells occurring in the context of a vigorous innate immune reaction emerges as a key factor responsible for the development of tumor antigen-specific adaptive immune response that contributes to the eradication of PDT-treated cancers. *Lasers Surg. Med.* 38:500–508, 2006. © 2006 Wiley-Liss, Inc.

Key words: acute phase response; adaptive immune response; efferocytosis; innate immunity; inflammation; photodynamic therapy

INTRODUCTION

The mechanism of tumor eradication by photodynamic therapy (PDT) comprises a highly complex interplay of responses at both local and systemic level. At the time of inaugural clinical studies it was assumed that the positive outcome results simply from the light-instigated direct tumor cell cytotoxicity of porphyrin photosensitizer preferentially retained in tumor tissue [1]. However, it became increasingly evident that indirect cytotoxicity owing to host-related factors has a decisive role in PDT-mediated tumor destruction [2]. It is now clear that tumor vasculature is a salient target of PDT and that multiple elements of host response are also important participants [3,4].

Collapse of PDT-treated tumor tissue with necrosis and eschar formation occurs within 24 hours with most photosensitizers. However, clamping subcutaneous tumors (completely occluding blood vessels leading to and from the tumor) immediately after Photofrin-PDT prevents the ablation of these lesions [4]. No visible signs of damage in these tumors were detectable when the clamps were removed 18 hours later. This experiment done with a mouse model demonstrates that the interaction with the host is indispensable for PDT-mediated tumor destruction.

A large body of evidence gathered mostly from animal models affirms that the outcome of tumor PDT is critically dependent on the contribution from the host [5]. Foremost, large differences in PDT-mediated cure rates can be seen between tumors implanted from the same cohort into syngeneic immunocompetent and immunocompromised mice [6]. Dramatic alterations in tumor cure rates were achieved by a wide variety of interventions modulating specific host response elements after PDT treatments. These include the depletion or impairment of the function of different immune cell types and platelets, neutralizing the action of cytokines and chemokines, blocking the expression of leukocyte adhesion molecules, and modulating the activity of plasma cascade systems (complement, coagulation, fibrinolysis) [5].

There have been important advances in the understanding of PDT-associated host response in recent years. To a large extent, this has been inspired by newly acquired general knowledge about innate immunity, inflammation, and adaptive immune response. This has helped us in attempting to find the answers to questions on why PDT elicits a vigorous host response, how it develops and what are its characteristics, what is its relevance to the clinical outcome, and how can it be exploited for improving therapeutic gain.

CAUSE AND PURPOSE OF PDT-ASSOCIATED HOST RESPONSE

An insult localized to a tumor is perceived by the host not much differently than a harm sustained in any other part of

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the body. Treatment of tumors by PDT results in an oxidative stress at the targeted site associated with a wide range of photooxidative lesions produced in the membranes and cytoplasm of cancer cells, tumor vasculature, and other stromal elements [7]. This rapidly induced massive tumor tissue injury delivered by PDT is experienced by the host as a local trauma threatening the integrity and homeostasis at the affected site [4]. Hence, the host is provoked to launch canonical responses that have evolutionary evolved for dealing with localized injury. The primary purpose of the intervention of the engaged host-protecting mechanisms is to prevent the spreading of tissue damage, contain the disrupted homeostasis, remove the dead and damaged/ altered tissue, promote local healing, and restore tissue function at this site. These tasks are carried out by the inflammatory response engendered with a crucial input from the innate immune system.

Importantly from the therapeutic endpoint, in its role of isolating and eliminating the incapacitated irreparable tissue the elicited host response amplifies the PDT-instigated eradication of tumor cells. This can be further augmented by the development of adaptive immune response recognizing PDT-treated tumor as its specific target [4]. On the other hand, host mechanisms mobilized to prevent excessive and harmful effects of the prolonged inflammatory/immune responses and advance healing may promote the recurrence of PDT-treated tumors [5].

RECOGNITION OF PDT-INDUCED INSULT

The key role in the initial phase of PDT-instigated host response is played by the innate immune system whose recognition arm is responsible for detecting the existence of an insult manifested by the appearance of "altered-self" [8]. Consistent with the danger model [9,10], which has received wide recognition in recent years, host-protective mechanisms are called into action by an alert system sensing alarm/danger signals from the tumor tissue distressed/injured (i.e., altered) by PDT treatment [5,8]. It appears that PDT is particularly effective in generating very rapidly an abundance of danger signals.

Endogenous Danger Signals Generated by PDT

In analogy to the adaptive immune response, innate immunity is extremely selective but instead of recognizing every possible antigen it rather focuses on a definite election of highly conserved structures present in potentially noxious substances. According to the danger model, distress and damage-related self molecules present damage-associated molecular patterns (DAMPs) [10]. In comparison, danger signals revealing the presence of nonself associated with infection are presented as pathogen-associated molecular patterns (PAMPs) [11]; these are molecular motifs shared by a large group of pathogens (such as lipopolysaccharide, flagellin, or peptidoglycans).

In principle, DAMP can be a breakdown product of damaged biomolecules or any molecule that is abnormally exposed, misfolded, or displayed at a wrong location because of injury. We have proposed that three major groups of DAMPs can be generated by PDT: cell-derived

molecules, extracellular matrix degradation products, and extravasated plasma proteins [5,8].

Cells sustaining PDT insult manifest molecular changes on their surface, as well as release of cellular material. Such exposure of molecules that are normally contained exclusively inside cells represents a potent danger signal. Heat shock proteins (HSPs), which are well-characterized as DAMPs [10], were recently shown to rapidly translocate to the cell surface following PDT and are also released from PDT-treated cells [12]. Other DAMPs in this category are degradation products of cellular membranes (lysophospholipids and arachidonic acid metabolites) that are abundantly released at PDT-treated sites [4,13,14]. In addition, a large number of DAMP candidates are among intracellular molecules released massively from cells rendered necrotic by PDT treatment [15].

Direct damage induced by PDT in extracellular matrix, whose constituents have the capacity to bind photosensitizer molecules [16,17], can result in the degradation of this supporting scaffold. This will release fragments of fibronectin, hyaluronan, collagen, and laminin that are known as danger signals [10,15,18,19].

Tissue injury causing the breakdown of blood vessels leads to the escape of plasma proteins normally contained only within the circulation. Fibrinogen and other displaced (extravasated) plasma proteins are also recognized as danger signals [20]. Increasing accumulation of extravasated fibrinogen was detected in PDT-treated tumors [5]. Intratumoral injection of fibrinogen after PDT treatment enhances PDT response [5], which supports the possibility that extravascular localization of this plasma protein promotes the development of host response against the treated tumor.

Receptors Recognizing PDT Damage

Sensors specialized in the detection of DAMPs and PAMPs have developed as the recognition arm of the innate immune system. These pattern-recognition receptors (PRRs) are non-clonal germline encoded molecules, which is in contrast to somatically generated clonally rearranged antigen receptors of adaptive immunity [21]. Another important distinction compared to the adaptive immunity is that the effector cells are activated and perform their function immediately upon the engagement of PRR rather than after they have proliferated.

Functionally, there are signaling, endocytic, and secreted PRRs [22]. Prominent members of the first group are Toll-like receptors (TLRs). Heat shock protein 70 (HSP70), which appears to be a major PDT-generated danger signal, was shown to bind TLR2 and TLR4 on macrophages co-incubated with PDT-treated tumor cells and this triggers signaling pathways responsible for activating nuclear transcription factor NF- κ B-mediated expression of various host response genes including those encoding TNF α and complement proteins [12,23].

Endocytic PRRs, for instance macrophage scavenger receptor, recognize PAMPs on invading microorganisms and DAMPs on dead cells/debris and mediate phagocytic removal of these targets [22]. They can be expected to have

an important function in the disposal of cells killed by PDT, but their roles have not yet been specifically investigated.

Secreted (soluble) PRRs, found in serum and tissue fluids, bind to PAMP- or DAMP-expressing structures and serve as opsonins flagging them as targets for the effector arm of host response [10,21,24]. There is burgeoning evidence of the involvement of the prominent members of this group, recognition members of the complement system and pentraxins, in tumor response to PDT. Tumor and endothelial cells treated by PDT were shown to become opsonized by complement protein C3 and its opsonizing fragments [25]. Another recognition molecule of the complement system, mannose-binding lectin (MBL) was found to accumulate in PDT-treated mouse tumors [26]. Pentraxins serum amyloid P component (SAP) and PTX3 were also detected to accumulate in mouse tumors treated by PDT [26].

INNATE IMMUNE AND INFLAMMATORY RESPONSE ELICITED BY PDT

Host-protecting responses elicited following the detection of PDT-generated danger signals are orchestrated by the innate immune system, and are characterized by the activation of two major effector processes, inflammation and acute phase response. The key element of the immediate response, inflammation, is instigated by the engaged soluble PRRs and PRRs on professional sentinels (mast cells and macrophages) and other cells. The strategy employed at this stage is to secure the affected site by targeting its vascular supply network and then to neutralize the focal source of danger signals by eliminating the injured cells. It should be emphasized that in this phase the nature of the host reaction is not strictly tumor-specific (would occur also with PDT treatment of normal tissues), but the intensity of some events (many danger signal molecules are overexpressed in tumors) and their consequences could be very different.

The Inductive Phase and Vascular Attack

The inductive phase of inflammation, mounted during PDT light treatment or immediately thereafter, is associated with a prompt formation/release of pro-inflammatory mediators (such as arachidonic acid metabolites, histamine discharged from mast cells, and complement anaphylatoxins) and rapid expression of genes encoding inflammatory chemoattractants, cytokines, leukocyte adhesion molecules, degradative enzymes, and other mediators [4]. Some of these mediators (e.g., cytokines) can be released from cells sustaining injury by direct PDT damage [27,28]. Their main tasks are to secure the recruitment of neutrophils and other inflammatory cells to the PDT-treated site (tumor) and to convert the tumor vascular endothelium from non-thrombotic non-adhesive barrier between the blood and tumor tissue to a pro-adhesive surface for inflammatory cells that is permeable for blood constituents.

A key role in the instigation and promotion of these inflammatory events is played by the proteins of the complement cascade, which is a central humoral

effector system of innate immunity. Accumulation and activation of complement proteins in PDT-treated tumors as well as systemic complement activation after PDT has recently been documented [29–31]. Moreover, evidence was obtained of the upregulation of key complement genes (C3, C5, and C9) in PDT-treated tumors that was traced to immune cells (primarily macrophages) invading the tumor after therapy [23]. Localized availability of complement proteins secured in this way may be critically important for sustaining PDT-induced host response. Blocking complement activity has a negative impact on PDT-mediated tumor cures (as shown by the results presented in Fig. 1), which demonstrates that complement system contributes to the eradication of treated tumors.

Complement anaphylatoxins C3a and C5a, as well as other inflammatory mediators (many of them generated secondary to complement activation) that are massively released from PDT-treated tumors [29,32], incite a rapid accumulation of large numbers of neutrophils in the treated lesions [33–36]. Principal causes for the extensive vascular injury observed in PDT-treated tumors include the assembly of lytic membrane attack complexes of complement on the vascular endothelium [29] and release of a variety of oxidants and proteases by adherent invading activated neutrophils. The inflicted disruption of endothelial integrity together with the degradation of basement membrane and matrix components occurring during acute inflamma-

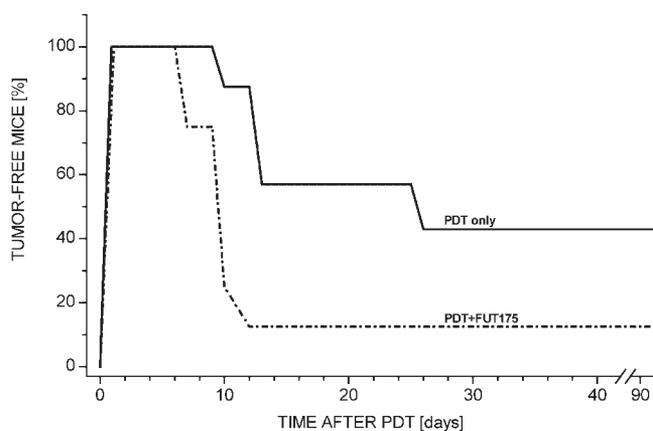


Fig. 1. Blocking complement activation reduces the cure rates of PDT-treated tumors. Subcutaneous FsaR fibrosarcomas (6–8 mm in largest diameter) growing subcutaneously in syngeneic C3H/HeN mice were treated by PDT (benzoporphyrin derivative 2.5 mg/kg i.v. followed 3 hours later by 100 J/cm² of 690-nm light). FUT-175 (BD Biosciences, Ontario, Canada), an inhibitor of the classical and alternate pathways of complement activation, was administered at 20 mg/kg i.v. immediately after PDT light. Mice were thereafter monitored for tumor growth, and no sign of palpable tumor at 90 days post-therapy qualified as a cure. In the absence of PDT, FUT-175 treatment had no detectable impact on tumor growth. Treatment groups consisted of eight mice. The difference in tumor response between PDT only and PDT+FUT-175 groups is statistically significant ($P < 0.5$; log-rank test).

tion often results in an increased vascular permeability and microvascular hemorrhage [37] as observed in the vasculature of PDT-treated tumors using many photosensitizers including Photofrin [38]. These events usually trigger plasma proteolytic cascades involving the activation of the coagulation system associated with platelet aggregation and vasoconstriction and lead to a complete vessel occlusion or microvascular collapse frequently described in PDT-treated tumors [39]. Modulation of the coagulation cascade activity and fibrinolysis also impacts markedly the outcome of PDT (as seen in Fig. 2), which confirms the participation of this humoral effector system of innate immunity [40] in the anti-tumor effect of PDT. Other elements, including the activity of nitric oxide [41] and events such as ischemia-reperfusion injury [42] can have important roles in the vascular effects of PDT [4].

Therefore, although the described events in the vasculature of PDT-treated tumors can be prompted by direct photooxidative lesions inflicted in endothelial cells, they can be also effectively initiated by PDT damage produced in perivascular regions of tumor tissue that instigates chemotactic gradients across the vascular endothelium. Thus, the vascular dysfunction in PDT-treated tumors can result from action of the inflammatory component of the induced host response whose purpose is to prevent spreading tissue injury with its harmful effects from the affected site by isolating and destroying its source (damaged tumor tissue). The latter is achieved by the attack on irreparably injured tumor cells by extravasating inflammatory cells.

Dead Cell Disposal

The next key task of the host-protecting response is the disposal of dead cells and the debris that has been created at the treated site. This errand is particularly important with PDT-treated tumors, because with this modality the host is suddenly faced with a burden of a large number of dead cells since the majority of cells in the targeted lesions are killed or dying by either necrosis or apoptosis within hours after treatment. Moreover, large numbers of dead neutrophils and other inflammatory cells that invaded the PDT-treated tumor and died after performing their function are also present. A swift nonphlogistic removal of all these corpses (particularly necrotic) is essential because their continuing presence would be a serious threat perpetuating active inflammation and impairing tissue integrity [43].

Efferocytosis (effero meaning “carry to the grave”) [44], evolutionarily highly conserved process of the disposal of dead cells, is secured by their recognition and phagocytic removal mediated by the innate immune system. Innate immune effectors serving as efferocytes are primarily macrophages, but other professional phagocytes (immature dendritic cells (DCs)) and any other tissue cell can also perform this function. Necrotic and apoptotic cells display “eat me” signals in form of de novo expressed molecular patterns (for instance, apoptotic cell-associated molecular patterns, ACAMPs) that are recognized by specialized innate immune PRRs [45]. With regards to PDT-treated tumors, the most prominently involved appear to be soluble

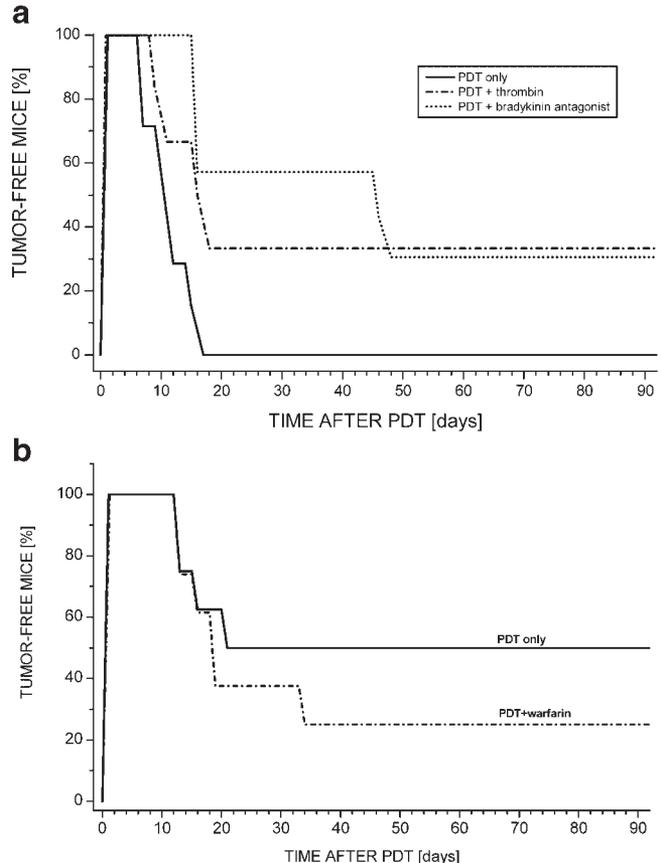


Fig. 2. The role of plasma coagulation system in tumor response to PDT. Squamous cell carcinoma SCCVII tumors growing subcutaneously in syngeneic C3H/HeN mice were treated by PDT (Photofrin 10 mg/kg i.v. followed 24 hours later by either 120 J/cm² (a) or 220 J/cm² (b) of 635-nm light. Mouse thrombin (Sigma, St. Louis, MO) at 8 units/mouse, bradykinin B2 receptor antagonist (Sigma B6029) at 4 mg/kg, and anti-coagulant warfarin at 10 mg/kg were administered i.v. immediately after PDT light treatment. The treatment responses were determined as described for Figure 1. Treatment groups consisted of seven to nine mice. The differences in tumor response between PDT only group and all PDT plus coagulation modulator groups is statistically significant ($P < 0.5$; log-rank test). Although the contrasting effects of the agents tested in (a) and (b) necessitated the use of two different PDT doses (possibly introducing variations in host response parameters), the results reveal that the coagulation system is an important participant in PDT-induced host response with a complex impact on the therapy outcome.

PRRs including complement proteins (C3, MBL, and possibly C1q and ficolins) and pentraxins (C-reactive protein, SAP and PTX3) [26]. The respective recognized ACAMPs likely include cell surface-exposed phosphatidylserine, oxidized membrane lipids or their altered glycosylation patterns [44,45]. Dead cells opsonized with PRRs are made more appetizing for the efferocytes, because they can bind them with their receptors dedicated for dead cell

ingestion [44,46]. Known to belong to this category are the scavenger receptor family, Fc receptors, phosphatidylinositol receptor, and complement receptors, such as CR3, CR4, gC1qR, and CD91-calreticulin [44,47]. Their role in the context of PDT response remains to be fully investigated.

As an insurance against engulfment by phagocytes, viable cells display on their membranes “don’t eat me” signals (also called self-associated molecular patterns, SAMPs) that help avoid unnecessary self-destruction by the innate immune system [46,48]. One group of SAMPs are proteins that negatively regulate complement activation. We have detected a decrease in the expression of this class of proteins, including decay accelerating factor (DAF, CD55), protectin (CD59), and mouse-specific Crry, on mouse tumor cells following PDT *in vivo* (Cecic and Korbek, unpublished results). This effect of PDT can facilitate the deposition of complement molecules on treated tumor cells and consequently their removal by phagocytes.

Efferocytosis occurring following treatment of solid tumors by PDT is one of the key events in the elicited host response. In addition to securing rapid clearance of the inflamed mass, it has a direct influence on the subsequent resolution phase of the inflammatory process and appears to have a critical impact on the development of adaptive immune response against PDT-treated tumors (as will be elaborated below).

Acute Phase Response

Optimal execution of host-protecting reaction triggered by PDT requires a systemic mobilization of the host’s resources. This is fulfilled by the orchestrated acute phase response, which is a dynamic homeostatic process that can be regarded as a stress response at the level of the organism raised for coping with tissue injury [49,50]. The induction of acute phase response is primarily mediated by IL6, which is known to be released following tumor PDT [34]. Systemic changes (distant from PDT-treated site) comprising the acute phase response include increased or decreased plasma concentrations of various proteins (acute phase reactants), enhanced hormone production by adrenal and pituitary glands, and increased blood leukocyte levels. Acute phase reactants have a wide range of activities implemented for optimizing and regulating the course of host response while minimizing its detrimental effects; some of them facilitate efferocytosis (pentraxins, complement proteins), others prevent excessive activity of inflammatory enzyme cascades (proteinase inhibitors), or promote wound healing (fibrinogen) [49,50].

The first report on PDT-associated acute phase reaction was from a study of normal mouse peritoneum treatment [51]. The authors described the induction of leukocytosis (due mostly to elevated neutrophil counts) and a surge in the levels of the prototypic acute phase reactant in mice, SAP. Subsequent investigations by Sluiter and co-workers and by our team have established the existence of a strong neutrophilia (with more than fivefold rise in the absolute number of circulating neutrophils in some cases) following PDT treatment of rat and mouse tumors, respectively

[29,32,35,52]. This acute phase phenomenon reflects rapid and massive recruitment of neutrophils (from the bone marrow and other non-circulating pools) and their accumulation and activation in PDT-treated tumors where they become engaged as inflammatory/immune effector cells [35,36].

Our findings revealed that neutrophilia in mice bearing tumors treated by Photofrin-based PDT is characterized by two distinct phases, an early phase culminating about 2–3 hours after PDT light treatment and advanced phase that peaks around 8–10 hours after PDT light; subsequently, the intensity of neutrophilia declines and becomes largely resolved by 24 hours post-PDT [29,35]. The results suggest that the neutrophilia is regulated through a programmed release of a succession of chemotactic mediators, with prostaglandins, leukotrienes, IL6, and histamine acting throughout this response, while others get involved either in the early phase (IL1 β , TNF α) or in the advanced phase (complement anaphylatoxins, thromboxane, G-CSF, IL10, coagulation cascade components). Chemokines and many other mediators acting directly or indirectly are also involved [4,29,53]. The two phases of PDT-induced neutrophilia reflect different roles of neutrophils in the early phase of the elicited host response (contributing to the destruction of tumor tissue) and in the advanced/resolution phase (downregulating inflammation, healing). Thus, depleting neutrophils from the host mice immediately after tumor PDT impacts negatively the therapy outcome [54] while delaying the depletion of these cells after PDT produces an opposite effect (Sun and Korbek, unpublished results).

The extent of PDT-induced neutrophilia is markedly less pronounced in adrenalectomized host mice compared to normal hosts [55]. This reveals the engagement of the adrenal-pituitary axis, one of the hallmarks of acute phase response, with the release of adrenocortical hormones that contribute to the neutrophilia expression.

We have recently obtained evidence of the increase in serum levels of major acute phase reactants in mice bearing PDT-treated tumors, including SAP, C3 protein, MBL, and PTX3 [26,31]. These proteins were found to accumulate in PDT-treated tumors, which is consistent with the idea of their interaction with tumor cells within the process of efferocytosis and the participation in other host response events.

Resolution of Inflammation

Unless properly and timely resolved upon the execution of acute inflammatory program, persistent inflammation can lead to chronic inflammatory and autoimmune disease. In analogy to the general nature of the inflammatory response, the resolution program of PDT-associated inflammation involves a switch from the production of pro-inflammatory to anti-inflammatory intercellular messengers. This occurs after the elimination of the source of the threat (PDT-injured tissue) and is linked with the programmed death of invading leukocytes by apoptosis and their removal together with dead resident cells [56].

Upon engulfing apoptotic cells, macrophages become reprogrammed starting to produce mediators that suppress inflammatory response and promote tissue repair [37,56]. Anti-inflammatory and immunosuppressive cytokines IL10 and TGF β are probably best characterized among these mediators. Both of them negatively regulate pro-inflammatory signaling by targeting NF- κ B, TGF β through its effect on mitogen-activated protein kinases (MAPKs) [57] and IL10 by augmenting the expression of I κ B α (cytoplasmic inhibitor of NF- κ B) [58]. Acting through Smad signaling pathway, TGF β promotes cell differentiation and proliferation (replacing losses of resident cells) and matrix production/fibrogenic remodeling involved in healing [59,60]. Blocking the activity of TGF β and IL10 after PDT markedly augments the cure rates of treated tumors (Table 1). This suggests that postponing the resolution of tumor-destructive phase of PDT-elicited inflammatory response mediated by these cytokines may improve the eradication of treated lesions. In contrast, the neutralization of inflammatory cytokine IL1 β post-PDT reduces the cure rate of treated tumors [36], consistent with the contribution of the early phase of inflammation to the therapeutic outcome.

Secretion of another agent critical for repair and growth following pro-inflammatory injury, vascular endothelial growth factor (VEGF), is also triggered by phagocytes following corpse engulfment [61]. Increased expression of this angiogenic factor and a variety of survival factors including matrix metalloproteinases and cyclooxygenase-2 (COX-2) is observed after tumor PDT and it was shown that their inhibition improves the therapy outcome [62–64].

TABLE 1. The Role of TGF β and IL10 in PDT Response of Mouse FsaR Tumors

	Tumor cure rate
Experiment 1	
PDT only	12.5%
PDT + anti-TGF β	50%
Experiment 2	
PDT only	43%
PDT + anti-IL10	100%

Subcutaneous FsaR fibrosarcoma-bearing C3H/HeN mice were treated by either Photofrin-based PDT (Photofrin 7.5 mg/kg followed 24 hours later by 100 J/cm²) in Experiment 1 or mTHPC-based PDT (mTHPC 0.1 mg/kg followed 24 hours later by 50 J/cm²) in Experiment 2. Anti-TGF β mouse monoclonal antibody (R&D Systems, Inc., Minneapolis, MN) was administered i.p. (30 μ g/mouse), while anti-IL10 rabbit polyclonal antibody (PeproTech, Inc., Rocky Hill, NJ) was given i.p. (12.5 μ g/mouse); both were injected at 24 hours post-PDT light treatment. Treatment groups consisted of eight (Experiment 1) or seven mice (Experiment 2). Both effects of cytokine-neutralizing antibodies were statistically significant ($P < 0.05$ for difference compared to PDT only groups). The treatment with antibodies alone had no significant impact on tumor growth, while isotype control antibody treatments showed no significant effect on PDT response.

This reveals that the resolution of inflammation and healing that supports angiogenesis and stromagenesis at PDT-targeted tumor sites is associated with a risk of promoting the recurrence of treated tumors in case of their incomplete eradication. Selective blocking of the released pro-survival agents at this stage post-PDT may afford therapeutic gain.

ADAPTIVE IMMUNE RESPONSE AGAINST PDT-TREATED TUMORS

After the innate immune system has, acting in antigen non-specific manner, eliminated the perceived threat at the PDT-treated tumor site and secured the re-establishment of normal homeostasis, the mission of elicited host-response would normally be considered fulfilled. However, it is becoming increasingly clear that the execution of this host-protecting response uncovers tumor-specific antigens and leads to their presentation for the recognition by adaptive immune system. Moreover, in the course of dealing with PDT-induced tumor-localized injury the innate immune system has become capable of priming and instructing the adaptive immune system to develop a response recognizing the treated tumor as its specific target. It is now well-established that PDT elicits T-cell specific immunity against the treated tumor with the generation of immune memory cells that can be recovered from distant sites [4,7,65]. This tumor-specific immune response develops too late to contribute to the initial tumor ablation, but may have a critical role in the eventual therapy outcome by preventing recurrences by the elimination of cancer cells residues remaining viable after the initial PDT response [7,66]. The extent of cytotoxic T-cell infiltration in the tumor following clinical PDT was found to correlate with the effectiveness of the therapy [67].

We propose that the key factor responsible for the development of PDT-elicited adaptive immune response is a highly intensified phagocytosis of dead tumor cells occurring in the context of a vigorous innate immune reaction. As emphasized, massive numbers of necrotic and apoptotic tumor cells and their debris are generated within a short interval after PDT, which compels the host to an extensive engagement in the disposal of this burden. Intracellular antigens of dying tumor cells (normally hidden to immune surveillance elements) survive the phagocytic process and are, particularly at high antigen loads, effectively “cross-presented” to T-cells [68,69]. However, phagocytes (functioning also as antigen-presenting cells (APCs)) that have acquired these tumor antigens would not be able to stimulate strong immune responses and may even induce tolerance in the absence of appropriate co-stimulatory activity [70]. Immature DCs are potent phagocytes, but they need to mature in order to deliver co-stimulatory signals required for them to function as effective APCs. Exposing immature DCs to PDT-generated tumor cell lysates was shown to induce their functional maturation [71]. It was also demonstrated that DCs migrate to PDT-treated tumor cells and subsequently accumulate in draining lymph nodes [72].

Dead cells are almost invisible in any tissue because they are, upon displaying “eat me” flags, very quickly removed. However, the appearance of large numbers of dead cells in PDT-treated tumors may temporarily overwhelm the capacity of phagocytes to ensure their swift removal resulting in the retardation of this process with consequent prolonged exposure of tumor antigens. Impaired clearance of apoptotic cells appears to be a major cause for the development of autoimmune disease, such as systemic lupus erythematosus (SLE) [73]. Concurrent appearance in PDT-treated tumors of both necrotic and apoptotic cells, whose phagocytosis have distinct impacts on immune responses [74,75], may render significant complementary attributes securing a strident anti-tumor immune reaction.

Since phagocytes have multiple receptors they can use for ingesting dead tumor cells, the opsonins decorating the target corpses (such as complement and pentraxin proteins) will largely determine which receptor will actually be engaged. Relevance of the phagocytic receptor type involved for the efficacy of subsequent processing and presentation of tumor antigens has only begun to emerge, and it has become clear that this can be of a critical importance for the development of tumor-specific immune response [76]. It has recently become evident that various complement and pentraxin proteins which are important participants in dead cell disposal are upregulated following tumor PDT [26], but their individual roles in the link between dead cell removal and immune recognition of PDT-treated tumors remains undefined.

Evidence obtained with the studies on PDT-generated cancer vaccines indicates that direct effects of PDT on tumor cells enhances their antigenicity, since these cells or their lysates are potent inducers of anti-tumor immune response which is not the case with tumor cells exposed to other treatments (ionizing radiation, UV, freeze-thaw cycles) [71,72]. As suggested, PDT-mediated photooxidation of cellular molecules can generate novel tumor antigens [77]. Alternatively, neo or cryptic antigens may be revealed as a consequence of protein unfolding or membrane changes occurring after PDT treatment.

Surface expression of HSP70 and other HSPs induced by PDT on tumor cells and their release [12] appears to have a major role in the PDT-associated immunogenicity. The HSP70 expression level in cancer cells correlates with their immunogenicity [78], and its membrane expression on these cells elicits CTL-mediated tumor immunity [79]. The COOH-terminal domain of HSPs, which becomes uncovered upon PDT-induced surface expression of these proteins [12], contains the binding site for chaperoned endogenous client peptides. Such exposure will attract immune surveillance cells which have specific receptors for HSPs [80] and may provide a source of tumor rejection antigens [81]. Surface expression of HSP70 may facilitate the deposition of complement proteins on PDT-treated cells [72]. Binding of complement fragments to tumor antigens enhances their presentation to T and B lymphocytes [82]. Thus, the association of both HSPs and components of the activated complement cascade with proteins of PDT-treated tumor cells may create unique

conditions for highly productive presentation of tumor antigens. These two elements were suggested to be largely responsible for securing the effective tumor control following treatment with PDT-generated therapeutic cancer vaccines [72].

CONCLUSIONS

Host response elicited by tumor treatment with PDT is an integral component of the therapeutic impact of this modality. It is executed as a tightly regulated host-protecting reaction orchestrated by the innate immune system. The antigen-specific adaptive immune system becomes mobilized as a secondary event also promoted by the innate immune system. This adaptive immune response is not programmed as a part of the host defense mechanisms raised in response to the inflicted tumor-localized injury, but is analogous to the autoimmunity development manifested as a side-effect escaping the control of a strong inflammatory process. The increased understanding of these host response mechanisms provides valuable insights for achieving further improvements in the efficacy of clinical PDT.

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