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The immunosuppressive side of PDT†

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Abstract

Photodynamic therapy (PDT) is a promising novel therapeutic procedure for the management of a variety of solid tumors and many non-malignant diseases. PDT has been described as having a significant effect on the immune system, which may be either immunostimulatory or, in some circumstances, immunosuppressive. The immunosuppressive effects of PDT have nearly all been concerned with the suppression of the contact hypersensitivity reaction in mice. Here, we review the immunosuppressive aspects of PDT treatment and discuss some additional mechanisms that may be involved.

Introduction

Photodynamic therapy (PDT) is a promising therapeutic procedure for the management of a variety of solid tumors and many non-malignant diseases. PDT is a two-step procedure that involves the administration of a photosensitizing agent, followed by activation of the drug with non-thermal light of a specific wavelength.^{1, 2} After the absorption of photons the sensitizer is transformed from its ground state into the long-lived triplet state *via* a short-lived excited singlet state. The triplet state can undergo two different reactions: it can undergo electron transfer to form radicals that interact with oxygen to produce oxygenated products, or transfer its energy directly to ground-state triplet oxygen to form singlet oxygen – thought to be the main mediator of PDT. The first alternative is called type I reaction, the second type II reaction.¹ PDT-generated reactive oxygen species (ROS) subsequently lead to oxidative stress and membrane damage in the treated cells and consequently led to cell death. The anticancer action of PDT is a consequence of a low-to-moderate selective degree of photosensitizer uptake by proliferative malignant cells resulting indirect cytotoxicity, and a dramatic antivasculature action that impairs blood supply to the area of light exposure.^{3, 4} Therefore, the biological responses to the photosensitizer are activated only in the particular areas of tissue exposed to light. PDT as a treatment procedure has been approved by the United States Food and Drug Administration for use in endobronchial and endoesophageal treatment,^{5, 6} and also as a treatment of premalignant and early malignant diseases of the skin (actinic keratosis), bladder, breast, stomach and oral cavity.¹

PDT has been described as having a significant effect on the immune system^{7–9} which may be either immunostimulatory or, in some circumstances, immunosuppressive. PDT is

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thought to be particularly effective at stimulating an immune response against a locally treated tumor^{10, 11} for the following reasons. PDT has been shown to effectively engage both the innate and adaptive immune systems in the host's responses to cancer.^{12–15} PDT alters the tumor microenvironment by stimulating the release or expression of various pro-inflammatory and acute phase response mediators from the PDT-treated site.^{16–19} The body recognizes the presence of local trauma threatening the integrity of the affected site, and releases proinflammatory mediators to maintain homeostasis.²⁰ PDT thereby prompts a powerful acute inflammatory response, causing accumulation of neutrophils and other inflammatory cells in large numbers at the treated site that may attack tumor cells.^{17, 21} The activation of the complement system in particular has emerged as a powerful mediator of PDT anti-tumor effects.^{22–26} Complement not only acts as a direct mediator of inflammation, but also stimulates cells to release secondary inflammatory mediators, including cytokines IL-1 β , TNF- α , IL-6, IL-10, G-CSF, thromboxane, prostaglandins, leukotrienes, histamine, and coagulation factors.¹⁹

In addition to stimulating local inflammation, PDT acts systemically to induce a potent acute phase response. PDT may also assist in maturation and activation of dendritic cells (DC) and increase their ability to home to lymph nodes and efficiently present tumor antigens and prime lymphocytes²⁷ capable of destroying distant, antigen positive tumors.²⁸

Since every reaction, *e.g.* PDT activation of the immune system, causes a counter-reaction, it is not surprising that there are also reports suggesting that PDT may in addition induce various forms of immunosuppression.²⁹ The immunosuppressive effects of PDT, if significant, would put this therapy in the same group as other anti-cancer modalities (Table 1) that are known to lead to immunosuppression.

The majority of studies discussed in this review have looked at PDT-mediated immunosuppression with the aim of ascertaining whether it is local or systemic, adoptively transferable and antigen specific. Additionally, some studies investigated cytokines and the types of the immune cells involved in this phenomenon. Below we present the summary of major findings.

Contact hypersensitivity studies

Contact hypersensitivity (CHS) is a form of T cell-mediated immunity that was originally described in 1960.³⁰ It can be induced experimentally by painting hapten on the skin, and this process mimics the inflammatory reactions seen with poison ivy and with various drugs and industrial or household chemicals. It can also be induced by conjugating the immunizing protein to a hapten carrier and then injecting the complex.³¹ There are three critical events that must occur in generating a reaction: (i) sensitization, (ii) trafficking and (iii) elicitation. In the sensitization phase, a naïve subject is exposed to hapten, usually through the skin and usually no symptoms of exposure are evident. The trafficking occurs when the hapten that binds covalently to any cell-associated protein or extracellular protein is subsequently picked up by antigen presenting cells (APC) and the activated APC present antigens to T cells, which recognize the modified antigen as foreign. In the elicitation phase, local APCs present the hapten-protein to memory T cells which have been formed during sensitization stage. The T cells then recruit more inflammatory cells to the antigen deposition site.

The earliest reports of immunosuppressive effects of PDT date to mid-1980s when Elmetts and Bowen demonstrated that pretreatment of mice with hematoporphyrin derivative (HpD) photoradiation resulted in 50% suppression of contact hypersensitivity to 2,4-dinitrofluorobenzene (DNFB).³² The results indicated that PDT-induced inhibition of contact sensitivity was a sustainable phenomenon and that the immunosuppressive response

required photo activation of the porphyrin molecule and was associated with the development of suppressor cells that were not further defined.

A subsequent study by Lynch *et al.*³³ provided evidence that PDT can lead to systemic immunosuppression. In this study (and the earlier described study) the suppressor cells were eventually identified as macrophages. Moreover, the observed effect could be adoptively transferred by viable splenocytes from PDT-treated mice.

Musser and Fiel³⁴ evaluated a series of porphyrins for their ability to induce a systemic immunosuppression. They defined this process as one of the most common PDT side effects and found out that HpD and *meso*-tetra(4-sulfonatophenyl)porphine led to the development of immunosuppression, while when Photofrin II or *meso*-tetra(4-carboxyphenyl)porphine were used this reaction was not observed. The two latter PS led to delayed type immunosuppression.

The following study by Musser *et al.* reported in detail how anatomic site of PDT affects the development of PDT-associated immunosuppression.³⁵ They compared the laser irradiation of flanks and thighs in tumor-free and tumor-bearing mice. Photofrin-mediated PDT led to the development of immunosuppression but the effect disappeared when PDT was carried out on a subcutaneously implanted foil disc that prevented light delivery to deeper tissues. The irradiation of the thigh also did not result in PDT-mediated immunosuppression while the irradiation of the tumors showed reduced immunosuppression. They concluded that the PDT-mediated activation of anti-tumor immunity was able to overcome any immunosuppressive effects that PDT may have activated.

A study by Simkin *et al.*³⁶ examined the effects of benzoporphyrin-derivative monoacid ring (BPD-MA)-mediated PDT on CHS. Interestingly, they observed that when mice were given BPD-MA alone and kept under ambient light they exhibited significantly reduced CHS ear swelling responses upon antigenic challenge. The BPD monoacid ring B (BPD-MB) also strongly reduced the CHS response while BPD diacid ring A (BPD-DA) as well as BPD diacid ring B (BPD-DB) also lowered the CHS response but were less effective than the monoacid forms. A significant reduction in the CHS response was observed when BPD-MA or PDT was given 48 or 24 h prior to, on the same day, or 24 or 72 h after sensitization. However, the CHS response was unaffected if these treatments were given 96 h after sensitization or 24 h before. The authors compared those reactions with other photosensitizers including Photofrin, tin etiopurpurin, and zinc phthalocyanine but neither of them altered the CHS response. The authors concluded that the inhibition of CHS responses and therefore immunosuppression was most probably due to the potent photosensitizing activity of these compounds even under the ambient light conditions

Silicon phthalocyanine 4 (Pc4), a second generation PS, was examined for its immunosuppressive properties.³⁷ Tumor-bearing mice were treated with Pc4-PDT 3 days before sensitization and showed significant immunosuppression of cell-mediated immunity. The observed immunosuppressive reaction was dose dependent and it was suggested to be of a systemic nature as it was observed at the remote site to the laser irradiation. It was also suggested that immunosuppressive reaction was not mediated by immunosuppressive cytokines like TGF β or IL-10 since the antibodies against those cytokines could not reverse it.

The role of IL-10 in PDT-mediated immunosuppression was further investigated in two subsequent studies. In the study by Simkin *et al.*³⁸ the immunosuppressive effects of PDT with BPD were investigated in normal and IL-10-deficient mice. In this approach mice received total body irradiation with red light at the dose of 15 J cm⁻². The results revealed that PDT with BPD and whole body red light irradiation led to a significant reduction in

CHS compared with control animals. The immunosuppressive effects of BPD-PDT were reversed by the administration of rIL-12 as well as by anti-IL-10 antibody. Furthermore, when the experiments were carried out in IL-10 deficient mice BPD-PDT treatment did not lead to immunosuppression. The authors, contrary to the previous study, concluded that IL-10 plays a key role in BPD-PDT-mediated immunosuppression.

However, a year later Gollnick *et al.*³⁹ published a study with the definite conclusion that “IL-10 does not play a role in cutaneous Photofrin photodynamic therapy-induced suppression of the contact hypersensitivity response.” In this study the cutaneous Photofrin-PDT suppressed the CHS reaction but the administration of anti-IL-10 antibodies had no effect on CHS suppression. Moreover, the experiments in IL-10 deficient mice of BALB/c and B6 background revealed that cutaneous Photofrin-PDT did not lead to significantly different immunosuppression when compared to wild-type mice.

Additional attempt to define the antigen specificity and cell types involved in PDT-mediated immunosuppression was undertaken by Musser and Oseroff.⁴⁰ When mice were subjected to PDT with Photofrin at various times after PS administration there appeared to be no correlation between drug-light interval and immunosuppression. The immunosuppression was increased if the sensitization was performed at least 3 days after PDT and could last up to 28 days after treatment. This reaction was adoptively transferable and specific for DNFB antigen. The depletion studies revealed that CD4+T cells were responsible for observed effects.

Interestingly, van Iperen *et al.* in the study where rats were given an injection consisting of PDT treated lymph node cells found out that this approach led to decreased CHS reaction however it was non-specific.⁴¹ The CHS reaction was decreased when the mice were re-challenged with DNFB but also with 2,4,6-trinitrochlorobenzene (TNCB).

To investigate whether PDT-mediated immunosuppression may depend on the PS used for PDT two other PS, protoporphyrin IX dimethyl ester (PPIX) and aminolevulinic acid (ALA) have also been studied in mouse models. Systemic immunosuppressive effects of PPIX have been tested in CBA mice.⁴² In actual fact, it was the potential immunosuppressive effects of PPIX photoproducts generated in solution before injection that were the focus of investigation. It appeared that the degree of CHS suppression correlated well with the fluence delivered to PPIX solution prior to injection. The investigation of PDT generated photoproducts of PPIX revealed that they contained isomeric chlorin structures that were actually better photosensitizers and both photoproducts were equally good at suppressing CHS. The question whether ALA-PDT can lead to immunosuppression has been addressed in the study by Hayami *et al.*⁴³ The study found out that after topical application of 20% ALA and 40 J cm⁻² of visible light the CHS reaction after DNFB application was significantly suppressed. The authors attributed the observed effects to the fact that after ALA-PDT the number of epidermal Langerhans cells (LC) was decreased 1 day after PDT and they only recovered at 5 days after PDT. Additionally they observed that the LC migrated to the local draining lymph nodes after PDT, leaving the irradiated skin area temporarily LC free. This would create at least a 5 days window where the sensitization and trafficking phase of the CHS reaction could not occur and therefore mice would not be responsive to local DNFB application. When the 80 J cm⁻² of light was delivered, the CHS response to the antigen applied on untreated distant skin was also significantly suppressed, suggesting the development of systemic immunosuppression that also turned out to be DNFB specific.

The relative success in demonstrating PDT-mediated immunosuppression in murine models was recently followed by studies of this phenomenon in the clinical setting. In the clinical

study Matthews *et al.*⁴⁴ involved healthy, PPD-positive volunteers that had been subjected to ALA or methyl aminolaevulinate (MAL)-mediated PDT. The skin on the back of study subject was irradiated with 37 J cm^{-2} of 630 nm light with and without prior application of ALA. Adjacent, untreated areas served as immunologically intact control sites. The PPD (Mantoux) preparation of tuberculin protein was used to elicit hypersensitivity responses at the site of irradiation or non-irradiated areas. The study revealed that both MAL-PDT and ALA-PDT significantly suppressed Mantoux erythema (by 30% and 50%, respectively) as well as the diameter of the reaction (41% and 38%). Interestingly the red light alone also significantly suppressed the diameter (22%) but not degree of erythema (13%). The study however did not report whether the difference between PDT and red light alone was statistically significant as all statistics were provided *versus* unirradiated control.

Skin graft and other immunomodulatory studies

One approach used to investigate the immunosuppressive effects of PDT involved skin graft studies. In the 1985 paper Gruner *et al.*⁴⁵ treated murine tail skin with HpD and light and subsequently proceeded with the allogeneic grafting. The donor BALB/c mice received 1.25 or 2.5 mg of HpD and 24 h later light up to 80 J cm^{-2} was delivered. Thereafter, the irradiated skin pieces were transplanted to CBA mice (transplantation across H2^d-H2^k haplotypes). The longest prolongation of graft survival compared to untreated control was achieved when the graft tissue received 40 J cm^{-2} and the HpD dose was 2.5 mg. It was observed that HpD-PDT led to decrease in ATPase activity of epidermal LC and that as a result, they lost their stimulatory capacity allowing for significantly longer graft rejection time.

In a study by Qin *et al.*⁴⁶ the immunosuppressive effects of peritoneal PDT procedure were examined. The authors investigated whether it will lead to the prolongation of skin graft survival. The results showed that HpD-mediated PDT prolonged the skin graft survival by about 6 days compared to control, non-irradiated mice. Neither light alone, PS alone nor application of PDT after grafting, significantly affected the graft viability. The peritoneal PDT led to significant depletion of peritoneal lymphocytes and an anergic state of lymphocytes isolated from spleens. Additionally they observed that peritoneal PDT led to significant increase in macrophage activation and phagocytosis.

The ability to prolong the skin graft survival was also tested with BPD-mediated PDT.⁴⁷ In this approach the *ex vivo* skin sections from C57BL/6 mice were pretreated with different doses of BPD-PDT and subsequently grafted onto BALB/c mice (transplantation across H2^b-H2^d haplotypes). The BPD-PDT pretreatment of skin grafts prolonged the graft survival from about 9 days to about 16 days and the difference was statistically significant. Interestingly, the higher doses of BPD did not result in longer graft rejection times and the most effective regimen involved $0.25\text{--}0.5 \mu\text{g ml}^{-1}$ of BPD. This relatively low dose of PDT led to downregulation of MHC and costimulatory B7 molecules on the LC significantly inhibiting their ability to activate alloreactive T cells.

A similar concept of applying *ex vivo* PDT to prevent transplant rejection was tested in the setting of murine neural xenografts.⁴⁸ In this model cell suspensions from fetal mouse mid-brain were incubated with BPD and subsequently exposed to light. After PDT the cell suspensions were inoculated into dopamine-depleted striata of hemi-Parkinson rats model and allowed 4 weeks to settle in the new environment. The xenograft function was subsequently measured by behavioral rotation assay as well as *ex vivo* immunohistochemistry. The low dose of BPD (25 ng ml^{-1}) allowed for survival of 60% of xenografts while the high-dose of 250 ng ml^{-1} only for 20%. The control cyclosporine A therapy led to survival of 90% of xenografts and in the non-treatment group all xenografts

were rejected. Although PDT was inferior to cyclosporine A treatment its major advantage was the relative ease of xenograft preparation and administration when compared to daily cyclosporine A treatment.

PDT immunosuppressive effects were also tested in the setting of autoimmune disease, namely adjuvant enhanced arthritis in MRL/lpr mice.^{49, 50} This is a model of the human autoimmune arthritis found in the disease systemic lupus erythematosus (SLE). The transcutaneous BPD-mediated PDT (0.5 or 1.0 mg kg⁻¹ and 162 J cm⁻²) was delivered by tungsten halogen light filtered between 600–900 nm at 10 day intervals starting on the day of adjuvant administration. PDT treated mice showed a delayed onset of arthritis as well as reduced severity when compared to untreated animals. PDT also prevented the cartilage and bone tissue damage and the observed beneficial effects were attributed to the PDT selective destruction of adjuvant-activated lymphocytes in the circulation and/or joints.

The effects of PDT mediated by 4,5-dibromorhodamine methyl ester (TH9402) were also studied in the context of DC function by.⁵¹ In the *in vitro* model that mimics the *in vivo* effects, blood mononuclear cells (PBMCs) were treated with TH-PDT and subsequently co-cultured with allogeneic immature monocyte-derived DCs. After 24 h, the phenotype and T-cell stimulatory capacity of the DCs was assessed. The results revealed that after phagocytosis of TH-PDT PBMCs, DCs remained in their immature phenotype, produced significantly increased amounts of IL-10, and had a reduced stimulatory capacity.

Discussion

Despite several aforementioned reports investigating PDT related immunosuppression, the exact mechanisms of this reaction have not been truly elucidated. It is highly probable that the local inflammatory reaction caused by PDT induces at the same time a compensatory anti-inflammatory response that may serve to limit potentially dangerous over-active immune responses and thereby reduce collateral tissue damage. It is possible therefore that in the certain contexts, PDT-mediated inflammation may lead to active immunosuppression. This would explain why some authors report PDT immunosuppressive effects that are in stark contrast to widely reported PDT related stimulation of anti-tumor immune response. Since most of the reported immunosuppressive PDT effects are based on the CHS reaction, that involves the application of a hapten such as DNFB to skin followed by a re-challenge, it is possible that PDT treatment may lead to the creation of a specific local immune privilege.⁵² Because it is local, PDT immune privilege would be different from systemic tolerance, although on occasion would lead to systemic effects over time. That would explain the contradictory reports in the literature. Similarly, if PDT treatment leads to the creation of local privilege it would not lead to systemic antigen-specific tolerance. The graphical illustration of this concept is presented in Fig. 1.

Another reason to favor this possible explanation is the fact that most of the CHS experiments were performed in skin and skin, on occasion, can become an immunosuppressive niche, *e.g.* during the process of wound healing,⁵³ which shares features in common with repair process of PDT-damaged tissue.

Much has been said about PDT-induced cell death and inflammation⁵⁴ but under some conditions dying cells may lead to immune tolerance.⁵⁵ Especially, when the dying cells are the healthy cells of normal tissues. In the case of PDT, the collateral damage to the normal cells at the site of treatment may be substantial and PDT may even affect the cellular members of the immune response.^{56–58} An additional factor that weighs in is the mode of cell death. Studies have shown that dendritic cells that feast on necrotic cell remnants can efficiently activate both CD4+ and CD8+ T cells, while the apoptotic cells may only lead to

CD8+ T cell activation. Without the help from CD4+, CD8+ T cells may become anergic and tolerogenic.⁵⁹

It would be interesting therefore to investigate whether PDT-related cell death and immunosuppression would vary depending on the PDT regimen selected; that is, would cellular PDT that had a higher probability to induce apoptosis lead to stronger immunosuppressive effects compared to vascular PDT that predominantly causes tumor necrosis?

The emerging concept of PDT-activated immune response is largely based on the release of damage associated molecular patterns (DAMPs) from PDT-treated cancer cells.⁶⁰ One of the members of the DAMP family is the nuclear DNA-binding protein high-mobility group box 1 protein (HMGB1)⁶¹ that can bind to Toll-like receptors (TRL) and activate the immune response.⁶² However, there is also evidence that when ROS are involved, oxidative modification of these immunostimulatory molecules may occur to promote tolerance instead of the stimulation of immune response.⁶³ During apoptosis, mitochondria permeabilize to release cytochrome c, which leads to activation of caspases that subsequently cleave NADH dehydrogenase Fe-S protein 1 (NDuFS1), a component of complex I in the electron transport chain.⁶⁴ The resulting inhibition of complex I function induces the production of ROS from the mitochondria, which oxidizes a key cysteine residue in HMGB1, neutralizing its ability to promote immune responses.⁵¹ It is therefore entirely possible that the abundance of different ROS during PDT may act in the same manner on HMGB1 and on occasion induce tolerance.

Additionally, there is also strong evidence that dying cells, especially those dying *via* apoptosis have been reported to release immunosuppressive cytokines such as transforming growth factor β (TGF β) and IL-10.^{65–67} Although there are conflicting reports as to the role of IL-10 in PDT-mediated immunosuppression the role of TGF β in the context of PDT immunosuppression has never been investigated.

Another possible mechanism by which PDT may lead to immunosuppression is by interfering with the maturation process of DC. There is significant evidence that PDT itself can cause a post-treatment increase in VEGF levels that correlate with a worse treatment outcome.⁶⁸ In addition to its pro-angiogenic properties, VEGF also has immunosuppressive properties as it inhibits DC differentiation⁶⁹ and is proposed to be one of the major mediators of disrupted DC differentiation.⁷⁰ *In vitro* studies have shown that VEGF blocks DC maturation, leading to the appearance of immature DC(iDC). Accumulating iDC can induce Treg and affect immune responses at the level of antigen-presentation and during the effector phase of T cells at the site of the tumor.⁷¹ Blocking VEGF augments normal DC differentiation and function,^{69, 72, 73} and treatment of tumor-bearing mice with an anti-VEGF antibody at a dose that did not block tumor growth directly but decreased serum VEGF levels by 90%, led to an increase in mature DC numbers, improved DC function and resulted in a pronounced decrease in tumor growth that was associated with an enhanced specific CTLs response. Thus, abnormal differentiation and maturation of DC *in vivo* mediated by tumor-derived soluble factors, mostly VEGF, likely plays a substantial role in preventing the effective priming of a productive, T cell-mediated anti-tumor immune response. It has never been however investigated if the observed VEGF surge after PDT impacts negatively on DC status and leads to post-PDT immunosuppression within the tumor milieu or whether any anti-VEGF therapies enhance PDT-mediated immune response.

Conclusions

Since PDT-mediated immunosuppression had been mostly tested in the CHS model it has been often compared to UV irradiation, which is known to suppress CHS in both elicitation and sensitization phases. Numerous mechanisms for this suppression have been proposed, including depletion of Langerhans' cells, aberrant regulation of cytokine secretion by both APCs and keratinocytes and down-regulation of co-stimulatory molecules on APCs. Based on the aforementioned literature examples one can conclude that to a certain extent PDT-mediated immunosuppression also follows the pathways described for UV irradiation. Nevertheless, the actual mechanisms behind this phenomenon are still poorly understood and additional studies are warranted. In addition, there are several other potential pathways that have not been yet explored that may greatly contribute to the development of PDT-mediated immunosuppression. Besides, the significance of PDT induced immunosuppression in particular in the tumor setting is still unclear and under-investigated and we summarized some of the additional mechanism that may operate in Fig. 2. Understanding those mechanisms may help to design combination therapies that may counteract them and lead to enhanced post-PDT immune responses, especially against cancers.

Biographies

Dr Pawel Mroz

Dr Pawel Mroz received his MD and PhD degrees from the Medical University of Warsaw, Poland. He joined the Wellman Center for Photomedicine at Massachusetts General Hospital, Harvard Medical School, as a postdoctoral research fellow in the laboratory of Dr Michael R. Hamblin in 2005. In 2008 he was appointed as an Instructor at HMS and Assistant in Immunology at MGH and Wellman Center. Dr Mroz has been investigating the variety of anti-tumor immune responses after photodynamic therapy; in particular, he has been investigating the role of T regulatory cells and tumor antigens in this process. Additionally, he has been involved in several projects evaluating the applications of new photosensitizers for PDT of cancer. He has received several awards for his research.

Dr Michael R. Hamblin

Dr Michael R. Hamblin is a Principal Investigator at the Wellman Center for Photomedicine at Massachusetts General Hospital and an Associate Professor of Dermatology at Harvard Medical School. He was trained as a synthetic organic chemist and received his PhD from Trent University in England. His research interests lie in the areas of photodynamic therapy for infections, cancer, and heart disease. In particular he has worked on covalent photosensitizer conjugates, induction of antitumor immunity by PDT, PDT for vulnerable

atherosclerotic plaque and antimicrobial photoinactivation *in vitro* and *in vivo*. He is also interested in basic mechanistic studies in low-level laser (light) therapy and its application to wound healing, traumatic brain injury, and hair regrowth. He has published over 120 peer-reviewed articles, over 150 conference proceedings, book chapters and international abstracts and holds 8 patents.

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Fig. 1. Potential mechanism that involved in PDT-mediated local and systemic immunosuppression. PDT tissue damage leads to release of various antigens that elicit activation of immune and regulatory responses. The local activation of the immune response after PDT may lead to DC translocation to the regional lymph nodes (LN) and subsequent presentation of antigens to naïve T cells. However, this activation may trigger counteraction that leads to the suppression of PDT-mediated immunity. The DC may not only deliver the antigen to LN in a stimulatory way but also in a tolerogenic manner. The activated T cells may be on the other hand prevented from functioning by T regulatory cells or immunosuppressive cytokines that may be locally secreted in the response to PDT insult and resulting inflammation. Tregs may also be responsible for observed systemic PDT effects. APC – antigen presenting cells, DC – dendritic cells.

Fig. 2.

Potential mechanisms of tolerance induction by PDT of tumors. PDT of tumor cells leads to apoptotic cell death that may release DAMPs, however ROS produced during PDT may inactivate the immunostimulatory potential of these molecules. Apoptotic cells may also release immunosuppressive cytokines like IL-10 or TGFβ or stimulate macrophages to release them. Immunosuppressive cytokines may in turn change CD4+ T cells into inducible Treg cells, while the lack of CD4+ T cell co-stimulation will result in generation of anergic CD8+ T cells. Additionally, high levels of VEGF released during PDT may affect the maturation of DC resulting in creating highly tolerogenic and immunosuppressive environment. Mφ – macrophages, DC – dendritic cells, iDC – immature dendritic cells, ROS – reactive oxygen species, DAMP – damage activated molecular pattern, VEGF – vascular endothelial growth factor, TGFβ – transforming growth factor β.

Table 1

Examples of potential immunosuppressive effects of selected anti-cancer therapies

Therapy	Immunosuppressive effects	Ref.
Radiation therapy	Lymphopenia	74
Chemotherapy	Lymphopenia	75
Surgery	Lymph nodes dissection	76
Steroids	Upregulation of TGF β production	77
	Impairment of NK-cell functions	
	Suppression of production of pro-inflammatory cytokines and chemokines	
	TH2-cell bias	
Imatinib	Impairment of DC differentiation or activation	78
	Alteration of TCR-mediated T-cell activation	
Impairment of CD8+ T-cell memory		
Taxanes	Inhibition of T-cell and NK-cell proliferation and activation	79
Photodynamic therapy?		