



REVIEW

The influence of photodynamic therapy on the immune response

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Summary Photodynamic therapy (PDT) is a clinically approved therapeutic modality used for the management of several types of tumors as well as non-malignant diseases. Most of the effects of this treatment regimen result from direct action of singlet oxygen and reactive oxygen species. However, accumulating evidence indicates that antitumor effects are also mediated by indirect stimulation of inflammatory and immune responses. These responses include rapid local infiltration of tumors by neutrophils and macrophages accompanied by systemic release of inflammatory mediators. This early response can initiate and translate into a more precise immune reaction that involves activation of specific T lymphocytes that seem to be necessary for the ultimate control of residual tumor cells. Although still incompletely understood, PDT can not only activate but also suppress the immune response depending on several variables. This review summarizes the influence of PDT on the immune response and discusses its importance in the management of human diseases.

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Abbreviations: 8-MOP, 8-methoxypsoralen; BCG, Bacillus Calmette-Guerin; ALA, 5-aminolevulinic acid; CD, cluster of differentiation; CHS, contact hypersensitivity; DBPMAF, Vitamin D₃-binding protein-derived macrophage-activating factor; DC, dendritic cells; EAE, experimental autoimmune encephalomyelitis; ECP, extracorporeal photochemotherapy; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophages colony-stimulating factor; GVHD, graft-versus-host disease; GVL, graft versus leukemia; HLA, human leukocyte antigen; HSP, heat shock protein; IFN, interferon; KC, keratinocytes-derived chemokines; MAC, membrane attack complex; MHC, major histocompatibility complex; MIP, macrophage inflammatory protein; MPO, myeloperoxidase; MS, multiple sclerosis; NK, natural killer; NO, nitric oxide; PDT, photodynamic therapy; PIT, photoimmunotherapy; SOD, superoxide dismutase; TAA, tumor-associated antigen; TGF- β , transforming growth factor- β ; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; UVA, ultraviolet A

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Introduction

Photodynamic therapy (PDT) is a minimally invasive therapeutic modality approved for the treatment of neoplastic and vascular diseases. It consists of (i) a photosensitizer that is applied topically or administered systemically; (ii) light in the visible range of electromagnetic wave, usually generated by laser sources and (iii) molecular oxygen, which is used in the photodynamic reaction to generate singlet oxygen ($^1\text{O}_2$) and reactive oxygen species. PDT is a two-step procedure: after local or systemic administration of a photosensitizer light of an appropriate wavelength is precisely delivered to the target tissue. Advanced fiberoptic systems enable light delivery to virtually any site in the body including brain and even small blood vessels.

PDT is frequently regarded as a dual specificity treatment. The selectivity is achieved by an increased photosensitizer accumulation within the tumor as compared to normal tissues and by the fact that illumination is limited to a specified location. The local nature of PDT is both a drawback and an advantage of this therapeutic modality. A limitation of PDT is that it cannot be a curative procedure for large and disseminated tumors. Nonetheless, even for advanced disease it can improve the quality of patients' life and prolong survival. For small and localized diseases, it can be a curative procedure with minimally invasive and excellent cosmetic results. Antitumor effects of PDT result not only from its direct action on tumor cells but also from its influence on the development

of inflammatory and antitumor immune responses. Several recent reviews extensively describe photosensitizers, basic photochemistry and mechanisms of antitumor effects of PDT [1–6].

The role of the immune response in the antitumor action of PDT

Early vascular effects induced by PDT

Direct endothelial cell damage accompanied by vessel contraction leads to exposure of basement membrane and vascular leakage that subsequently contribute to early events of PDT such as edema formation, platelet aggregation, thromboxane release and thrombus formation as well as activation of the complement cascade [7,8]. Moreover, PDT inhibits the release of nitric oxide (NO) from endothelial cells, thereby further contributing to vessel constriction [9]. The early vascular shutdown leads to ischemia-related cell death. The collapsed vessels may stay closed but in some cases there is a reoxygenation of the treated tissues soon after completion of tumor illumination. This latter effect can be facilitated by the release of vasodilating mediators such as NO, histamine and prostaglandins. In a poorly oxygenated environment, xanthine dehydrogenase becomes converted into xanthine oxidase which converts hypoxanthine into xanthine in a process accompanied by a release of large amounts of reactive oxygen species, mainly superoxide anion. This oxidative stress promotes

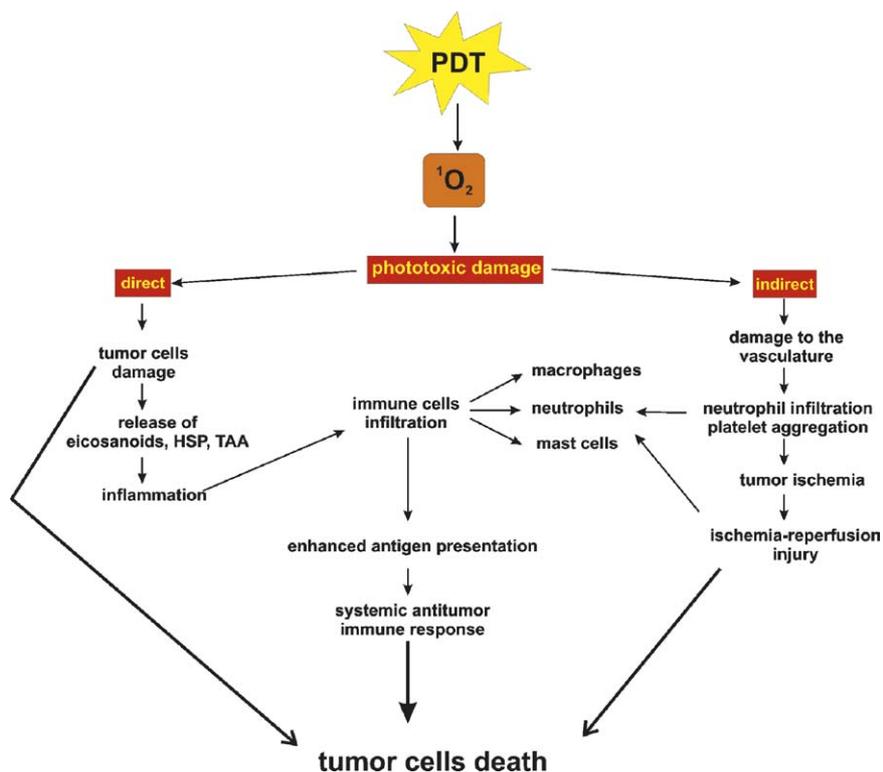


Figure 1 The mechanisms of antitumor effects triggered by PDT. PDT induces both direct and indirect antitumor effects. It can directly destroy tumor cells that undergo apoptosis and necrosis accompanied by the release of numerous mediators such as eicosanoids, heat shock proteins (HSP) and tumor-associated antigens (TAA). These mediators induce a non-specific, inflammatory response that is facilitated by the destruction of tumor vasculature. The inflammatory response is followed by a slowly developing adaptive immunity that can potentiate local antitumor effects and might possibly (at least in some experimental tumor models) induce systemic immunity.

complement activation and infiltration of a previously ischemic area with neutrophils and other inflammatory cells [10]. Therefore, after a brief period of ischemia a reperfusion injury occurs which further contributes to PDT-induced tumor cell death [11,12]. Administration of a bacterial superoxide dismutase (SOD) or inhibition of xanthine oxidase leads to attenuated antitumor effects of PDT [11,13]. Accordingly, inhibition of SOD activity by 2-methoxyestradiol potentiates antitumor effects of PDT in two murine tumor models [14].

Direct induction of tumor cell death potentiated by ischemia and ischemia-reperfusion injury is responsible for early tumor ablation. However, accumulating evidence indicates that these early events trigger inflammatory responses that seem important in achieving long-term tumor control (Fig. 1).

The influence of PDT on complement activation

As mentioned above PDT results in activation of the complement system. The activation of the

complement cascade, the major effector system of innate immunity, seems to play an important role in the initiation and orchestration of the PDT-induced response. Complement engagement is triggered by one of the three independent activation pathways, i.e., classical, alternative or lectin mediated. The likely pathway for complement activation after PDT is an alternative pathway as PDT can effectively activate a complement cascade in *scid* mice that do not have antibody-producing B cells [15,16]. Additionally, some recent studies indicate that activated platelets can also contribute to the activation and propagation of the complement cascade [17]. The generated cleavage products C3a and C5a are highly potent chemotactic factors that attract and activate neutrophils, macrophages, mast cells and T cells. Activation of a complement cascade and generation of membrane attack complex (MAC) on vascular endothelium of PDT-treated tumors is likely to contribute to the collapse of blood supply. Complement activation has been exploited in combination studies aimed at potentiating the antitumor effects of PDT. Treatment with zymosan, an activator of neutrophils, macrophages

and alternative complement pathway reduced the recurrence rate of PDT-treated tumors [18]. In contrast, treatment with heat-aggregated gamma globulin (complement activator through the classical pathway) was ineffective in achieving potentiated antitumor effects of PDT, thereby confirming that complement is activated via alternative pathway. Moreover, systemic complement activation with streptokinase had no detectable effect on complement deposition at the tumor site without PDT but it augmented the extent of complement activity in PDT-treated tumors. Neither zymosan nor streptokinase influenced the effectiveness of PDT in complement-deficient mice [18].

Induction of local inflammatory response following PDT

Not only initial vascular damage, ischemia and ischemia-reperfusion injury followed by platelet aggregation and complement activation are important in eliciting the early inflammation. PDT-mediated oxidative stress triggers a vast array of signal transduction pathways that induce apparently protective responses. These include expression of heat shock proteins, and transcription factors such as NF- κ B and AP-1 [19–21]. These two alone can induce the expression of dozens of cytokines, adhesion molecules, co-stimulatory molecules and immunologically important genes (Table 1). Additionally, photooxidative degradation of membrane lipids and generation of arachidonic acid metabolites are themselves potent inflammatory mediators that precipitate a rapid and strong inflammatory reaction [22]. These processes together with the release of histamine and serotonin from damaged vasculature induce a sequential arrival of neutrophils, mast cells and monocytes/macrophages that become activated and engaged in the tumoricidal activity (see below).

Interestingly, despite almost complete blood flow stasis leading to reduced wall shear stresses, PDT does not induce increased leukocyte adhesion to postcapillary venules within the area exposed to laser illumination [23]. Nonetheless, PDT induces leukocyte adhesion in microvessels of normal tissues, not directly exposed to PDT. Therefore, it seems that the inflammatory mediators released from PDT-treated tumors are capable of inducing expression of cell adhesion molecules in normal or minimally damaged endothelial cells but not in tumor blood vessels directly exposed to PDT. These unexpected observations are difficult to reconcile in terms of intense tumor infiltration by neutrophils, macrophages and mast cells observed within hours following PDT (see below).

Table 1 Selected target genes elicited by PDT-induced transcription factors AP-1 and NF- κ B.

Transcription factor	Target genes	Function of target genes
AP-1	VEGFD	Angiogenesis
	Fas, FasL	Apoptosis
	MMP1, MMP3, uPA, uPAR	Invasiveness
	EGF, HB-EGF, KGF	Proliferation
NF- κ B	GM-CSF, IL-1, -2, -6, -8, TNF	Inflammation
	Chemokines (IP-10, KC, MIP-1 α and β , eotaxin, MCP-1, RANTES)	Chemoattractants for leukocytes
	IL-1 α , IL-1 β , IL-1RA, IL-2, -6, -8, -9, -11, -12, -15	Interleukins
	IFN- γ , IFN- β	Interferons
	TNF, LT- α , LT- β , TRAIL, Fas	Tumor necrosis factor superfamily
	G-CSF, GM-CSF, M-CSF, VEGFC, PDGF	Growth factors
	ICAM-1, VCAM-1, P-selectin, E-selectin	Adhesion molecules
	MHC class I, B7.1, B7.2	Antigen presentation and co-stimulation
	Complement components, C-reactive protein, SAA, TF-1	Acute phase response
	COX-2, iNOS	Inflammation

Abbreviations: EGFR, epidermal growth factor receptor; FASL, FAS ligand; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte–macrophage colony stimulating factor; HB-EGF, heparin-binding EGF; ICAM, intracellular adhesion molecule; IL, interleukin; KGF, keratinocyte growth factor; M-CFS, macrophage colony stimulating factor; MHC, major histocompatibility molecule; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; SAA, serum amyloid A; TF, tissue factor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor; VEGF, vascular endothelial growth factor; VCAM; vascular cell adhesion molecule.

A likely explanation for these effects is that PDT induces different responses in different regions of the tumor. In areas proximal to the light source where the effects of PDT are the strongest, we might expect complete vascular collapse with blood flow stasis and no leukocyte attachment or recruitment. In distal regions, exposed to suboptimal laser illumination, there would be no endothelial cell damage but rather their activation followed by

the expression of adhesion and pro-inflammatory molecules and recruitment of leukocytes.

Systemic inflammation after PDT

While, the inflammatory response is considered an important priming event for the development of adaptive immunity it can also exert systemic adverse effects. Indeed, PDT itself induces a strong acute phase response dominated by neutrophilia and the release of various cytokines [16,24]. PDT-elicited, tumor-derived factors including C5a complement component, interleukin (IL) 1 β , IL-6, IL-10, tumor necrosis factor (TNF), granulocyte colony-stimulating factor (G-CSF), thromboxane, leukotrienes, histamine, prostaglandin E₂, clotting factors and a chemokine KC are all responsible for increase in the number of blood neutrophils [15]. Relatively high PDT doses (10 mg/kg Photofrin and 200–500 J/cm² light) confined to hind limbs of mice caused high level of lethality induced by a systemic response that resembled traumatic shock injury [25].

Such complications are probably irrelevant to most PDT applications in humans, where the illuminated area is too small to induce expression of mediators capable of inducing systemic toxicity. However, it was reported that patients undergoing intraperitoneal PDT develop a significant capillary-leak syndrome that requires accessory

monitoring and therapeutic procedures [26]. Increased systemic concentrations of IL-1 β , IL-6, IL-8 and IL-10 have been detected in patients undergoing extrapleural pneumonectomy followed by intraoperative PDT delivered to the entire thoracic cavity [27]. However, in both these studies the influence of surgical procedure has not been excluded as a possible source of systemic response.

The role of neutrophils in PDT

Neutrophils are among the first cells of the innate immune system to enter PDT-treated tumors (Fig. 2). These cells adhere to the vascular wall within 5 min after the start of PDT [28]. Their migration into the tumor is regulated by PDT-induced expression of E-selectin, chemokines (MIP-2 and KC) and possibly other mediators [15,18,29,30]. Neutrophil-derived myeloperoxidase (MPO) is detectable within tumors barely 2 h following PDT and by 13 h it increases to values almost 200-fold greater than in non-treated controls [29]. MPO increase has been calculated to account for a number of 9 millions neutrophils infiltrating every 100 mg of tumor tissue after PDT [29]. It is likely that phagocytic cells are attracted to the PDT-treated tumors to remove cell debris following treatment. These cells might also be a source for chemotactic and immunoregulatory factors necessary for further propagation of inflammatory

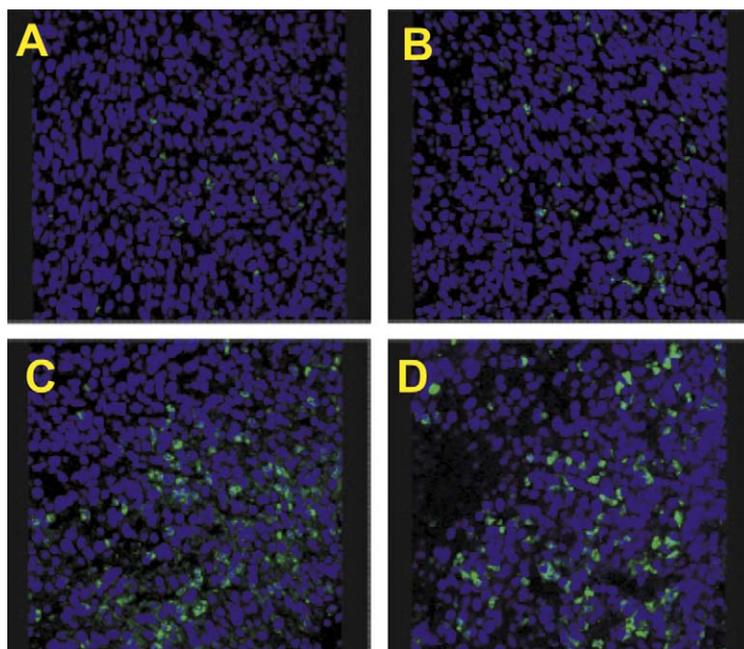


Figure 2 PDT induces recruitment of neutrophilic infiltrate. The immunofluorescence studies were performed on snap-frozen tumor sections obtained from C-26 tumors treated with Photofrin-based PDT. Fluorescently labeled neutrophils were detected with anti-Gr1 monoclonal antibodies. The nuclei of cells are stained with Hoechst. (A) Control tumors, (B) 4 h after PDT, (C) 8 h after PDT, (D) 24 h after PDT.

response [31]. Moreover, neutrophils can help PDT to damage tumor stroma that separates immune and inflammatory cells from neoplastic cells by extracellular matrix proteins [32,33], thereby rendering tumor cells "exposed" to their direct cytotoxic effects.

In different tumor models neutrophil depletion decreases the antitumor effectiveness of PDT [34,35]. Interestingly, reduced skin phototoxicity has been observed in leucopenic animals [36]. Similar effects are obtained after depletion of neutrophils with anti-Gr1 antibodies [35] or by blocking the activity of G-CSF [24]. Interestingly, a decreased tumor cure rates have also been observed after blocking some of the mediators of neutrophils, including complement, IL-1 β , IL-6, histamine, thromboxane, chemokines or xanthine oxidase, an enzyme induced by ischemia-reperfusion injury [15,29,37]. On the other hand, administration of G-CSF [35,38], granulocyte macrophage colony-stimulating factor (GM-CSF) [39] or anti-platelet serum that increases neutrophil accumulation in the tumor [40] improves tumor response rates of PDT. The results of these studies imply that neutrophils are not merely innocent bystanders, attracted to the site of PDT treatment to phagocytose tumor cells remnants but they actively participate in the destruction of tumor cells.

Other critical factors that determine the induction of an inflammatory response following PDT are light fluence and fluence rate [41]. These studies, however, did not confirm the role of the inflammatory cells in achieving maximal antitumor response. Optimally curative PDT regimen (high fluence [128 J/cm²], low fluence rate [14 mW/cm²]) produced minimal inflammation and yielded 70–80% tumor cures. Highest production of inflammatory cytokines and neutrophilic infiltrates were induced by suboptimal PDT regimen (low fluence [48 J/cm²] and low fluence rate [14 mW/cm²]) that produced only 10–20% tumor cures. PDT at high fluence led to a strong destruction of tumor vasculature while PDT at the same fluence rate but at low fluence produced virtually no vascular damage. The mechanisms of these effects have not been studied in detail. One can hypothesize that as leukocytes emigrating from blood vessels to tissues require interactions with endothelial cells mediated by selectins, chemokines and immunoglobulin-like molecules on the surface of inflamed endothelium [42], destroyed endothelial cells are unable to sustain effective tumor infiltration by neutrophils at vascular-damaging PDT regimens.

In clinical PDT, we are frequently confronted with much larger tumors than those induced in experimental animals. Light penetrating through

large tumors is subjected to a combination of variables that result in inhomogeneous light distribution to various regions of the tumor. Moreover, tumor tissue distant from the light source receives suboptimal light doses. Therefore, in clinical PDT proximal tumor regions receive light at high fluence rate and the illumination conditions are optimal for direct and anti-vascular effects of PDT. However, deeper regions of the tumor receive suboptimal PDT doses that might elicit inflammatory response. It should also be stressed that tumors in experimental animals constitute a much larger percentage of total body weight than tumors growing in humans. Therefore, systemic effects of PDT are possibly more pronounced in experimental setting.

The role of macrophages in PDT

PDT-treated tumor cells do not seem to be more susceptible for macrophage-mediated cytotoxicity in *in vitro* studies [43]. However, several studies indicated that PDT-treated macrophages exert potentiated phagocytic activity, produce increased amounts of TNF and NO and exert antitumor effects [44–47]. For example, macrophages isolated from PDT-treated SCCVII squamous cell carcinomas were almost five times more effective in killing tumor mice than those cells obtained from untreated tumors [48]. Moreover, macrophages were found to be indirectly stimulated for tumoricidal activity by photodynamically killed tumor cells [49].

The relevance of these *in vitro* studies for PDT efficacy *in vivo* is unknown. PDT induces an increased tumor infiltration with macrophages. Macrophages are efficiently accumulating most of the photosensitizers which might render them highly susceptible to PDT-mediated lysis. Moreover, PDT induces regions of hypoxia which have been shown to attract macrophages subsequently exploited for the production of pro-angiogenic factors [50]. Therefore, macrophages might also play a negative role. This issue merits further studies. Nonetheless, sublethal damage to tumor cells *in vivo* might render these cells more susceptible for macrophage-mediated cytotoxicity [51]. Indeed, macrophages were able to kill those tumor cells that recovered from PDT-induced damage [51]. It remains to be resolved what changes are induced in tumor cells that lead to their increased killing by macrophages. The influence of low doses of PDT in *in vitro* studies that lead to stimulation of selected effector mechanisms of macrophages might be relevant for the induction of tumoricidal activity of macrophages at deep tumor regions, where suboptimal tumor killing doses of PDT might be insufficient for tumor cells killing but might be

sufficient for the activation of tumoricidal activity of macrophages.

A circumstantial evidence for the role of macrophages in PDT has been provided by *in vivo* studies. Administration of silica, which is frequently used to inactivate macrophages *in vivo* [52], also markedly decreased the curative response of tumors to PDT [34]. It is important to note that administration of macrophage activating agents such as GM-CSF, Vitamin D₃-binding protein-derived macrophage-activating factor (DBPMAF) or Bacillus Calmette-Guerin (BCG) was effective in potentiating the antitumor effects of PDT [39,53,54].

The role of adaptive immunity in PDT

Development of an effective adaptive immune response requires prior engagement of innate immunity. The most important cells that link the non-specific and specific immune responses are dendritic cells (DCs) [55]. According to the "danger hypothesis" DCs act as sentinels that monitor the presence of infectious microorganisms, tissue stress, damage or transformation and elicit a specific and highly effective immune response [56–58]. Danger signals, in the form of microbial products, inflammatory cytokines or heat shock proteins (HSPs) act on DC precursors and influence their differentiation [59,60]. In the context of the antitumor immunity, tumor-associated antigens (TAA) are captured by DCs by several different mechanisms including ingestion of apoptotic tumor cells, fragments of necrotic tumor cells, or released soluble tumor antigens, especially associated with HSPs [61]. Antigen-loaded and appropriately stimulated DCs undergo final maturation and migrate to the local lymphoid tissues (lymph nodes, spleen or Peyer's patches) where they present TAA-derived peptides in the context of major histocompatibility complex (MHC) class I and II molecules to CD8+ or CD4+ T cells, being cytotoxic or helper lymphocytes, respectively [56,62]. Effective activation of T cells requires the presence of at least three signals: recognition of antigenic peptides presented by MHC molecules, co-stimulatory signals delivered by CD28 molecules and some members of tumor necrosis superfamily, *i.e.*, OX40, 4-1BB and fine tuning by local release of cytokines. Cytokines play a special role in regulating the type of an immune response that will develop after antigen presentation. Depending on the local milieu in the secondary lymphoid organ, helper T cells (Th) will differentiate into Th1, Th2 or regulatory subsets [63]. Th1 cells, through the secretion of IL-2, interferon- γ (IFN- γ) and TNF are responsible for development of cell-mediated immunity that involves the activity

of CD8+ cytotoxic T cells, macrophages and NK cells. Th2 cells, secreting IL-4, IL-5, IL-6, IL-10 and IL-13 drive development of humoral immunity. Different subsets of regulatory T cells (Th3, Tr1) secrete various combinations of IL-10, transforming growth factor- β (TGF- β) and other cytokines that downregulate an immune response [64]. During an effective immune response, activated CD4+ and CD8+ T cells are required to migrate from lymphoid organs to the tumor site where CD8+ T cells attack tumor cells directly, and CD4+ T cells dictate other cells of the immune system (including NK cells and macrophages) how to effectively destroy transformed cells [65,66].

Although direct tumor and vascular damage are responsible for most of the initial antitumor effects the long-term tumor control can be attained by concurrent activation of the immune response. During effective PDT, over 90% of tumor cells become lethally damaged within several hours of illumination. Thus, large amounts of tumor cell debris becomes available for phagocytic cells in a relatively short time interval. These cells become loaded with released tumor antigens and, in the inflammatory microenvironment, become activated to produce more inflammatory mediators, to process and to present tumor-derived antigens to host lymphoid cells (Fig. 3). In this aspect, it should be emphasized that not only dendritic cells and macrophages but to some extent also neutrophils and mast cells can become antigen-presenting cells [67,68]. Of these, only DCs are capable of getting into local lymph nodes to initiate an immune response. The remaining antigen-presenting cells can function locally to sustain an effective immunity. Interestingly, neutrophils from PDT-treated tumors express more MHC class II molecules on their surface [29].

Normally, apoptotic cells are swiftly cleared by phagocytic cells without inciting inflammatory or immune responses [69,70]. Phagocytosis of apoptotic cells can rather stimulate tolerance rather than immunity [71]. However, accumulating evidence indicates that "stressed" or oxidatively modified apoptotic cells may provide endogenous "danger signals" triggering inflammatory response [72,73]. Dendritic cells loaded with such stressed tumor cells elicit protective antitumor immunity [73,74]. Co-culture of DCs with PDT-treated tumor cells leads to their effective phagocytosis that promotes maturation of DCs, the release of pro-inflammatory cytokines and expression of co-stimulatory molecules [75,76]. Oxidative stress generates a variety of modified membrane lipids and lipid-protein adducts. PDT was shown to induce an almost instantaneous translocation

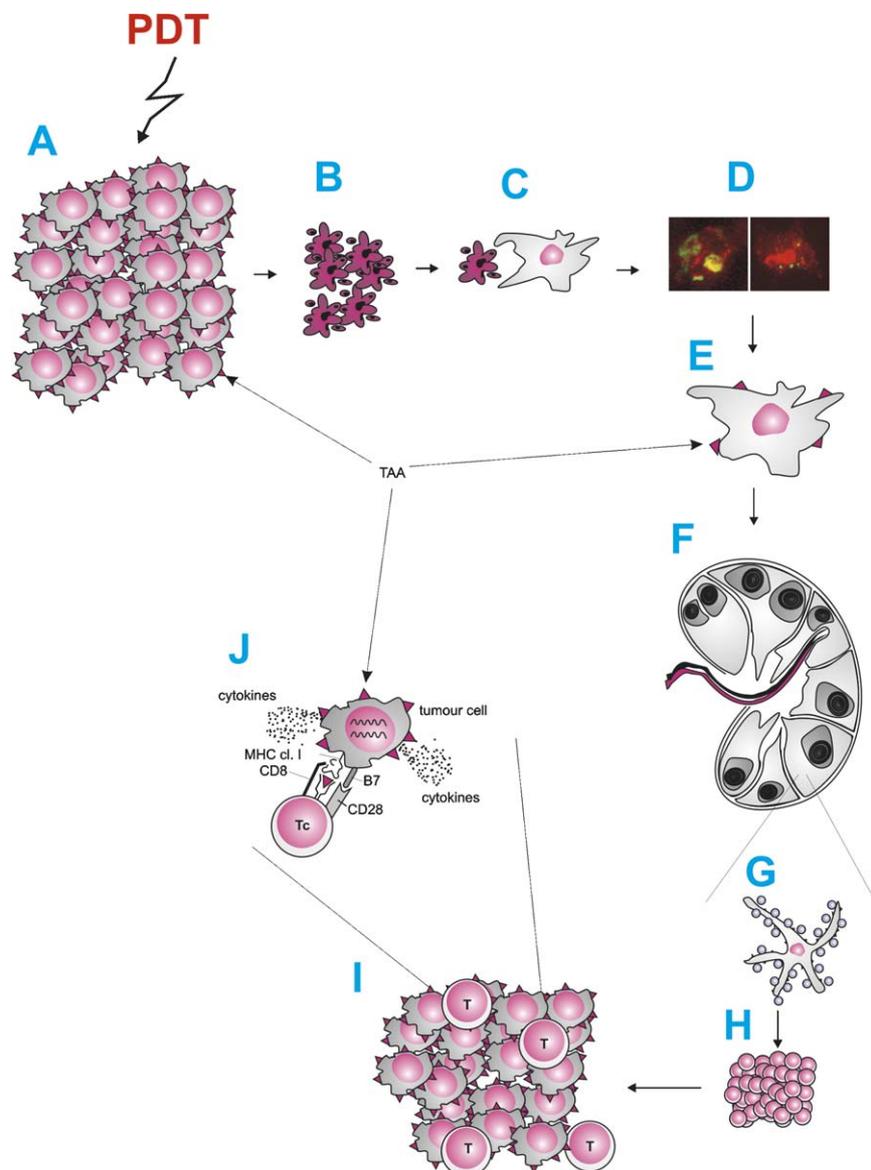


Figure 3 Mechanisms of PDT-elicited induction of antitumor adaptive immune responses. (A) Tumors are illuminated following administration of a photosensitizer. (B) Tumor cells undergo apoptosis and necrosis. (C) Some oxidatively damaged and apoptotic tumor cells are phagocytosed by immature dendritic cells that infiltrate peripheral tissues (including tumors themselves). (D) Immunofluorescent images of dendritic cells (stained in red with anti-MHC class II molecules) that phagocytosed CFSE-labeled (green) tumor cells or their fragments (C-26 colon adenocarcinoma) that underwent PDT procedure. (E) Dendritic cells, loaded with tumor-associated antigens (TAA), home to the local lymph node (F) where they present TAA to immature T cells (G). (H) Those T cells that effectively recognized TAA undergo clonal expansion and acquire the capacity to leave lymph nodes and get into peripheral tissues including the tumor (I). If these activated T cells can specifically recognize TAA presented by tumor cells, they can either directly kill tumor cells or release inflammatory cytokines that will recruit and activate other effector cells of the immune system (J).

of cellular HSP70 (and other heat shock proteins HSP60 and GRP94, but not GRP78) from the cytosol to the plasma membrane of tumor and endothelial cells [77]. The surface HSP70 expression was stable and persisted for at least 18h. This translocation was followed by the release of HSP70 from tumor cells. The released HSP70 could stimulate macrophages to secrete TNF in a Toll-like receptor

(TLR) 2 and TLR4-dependent manner. The finding that higher levels of HSP70 are found on apoptotic tumor cells is concordant with our hypothesis that PDT induces generation of stressed immune cells that might be efficiently taken up by professional antigen-presenting cells [75].

Studies in immunodeficient mice or in animals devoid of leukocyte subsets or immune effector

molecules after depletion with monoclonal antibodies are frequently used as experimental models to validate or disprove the role of the immune system in basic research studies. Such experiments have also unequivocally shown that the immune response participates in the antitumor effects induced by PDT. Selective depletion of CD8⁺ T cells, CD4⁺ T cells or NK cells showed no demonstrable effect on the initial ablation of PDT-treated tumors but promoted tumor re-growth [34,78].

At defined PDT conditions, long-term tumor ablation is observed in BALB/c mice inoculated with EMT6 mammary carcinoma cells. Identical PDT regimen in *scid* mice was not curative despite an initial complete response (no palpable tumor after treatment). Importantly, adoptive transfer of splenocytes obtained from normal, i.e., immune-competent mice that have been previously cured of their tumors, enabled a full tumor control in *scid* mice treated with PDT. Removal of CD4⁺ or CD8⁺ T cells partially abrogated development of protective immunity in *scid* animals [79]. Similar results were observed in nude mice that have no thymus and do not produce $\alpha\beta$ T cells [78]. The induction of immunity against PDT-treated tumors has also been shown in a weakly immunogenic murine MS-2 fibrosarcoma, where tumor-free animals rejected re-inoculated tumor cells 100 days following curative PDT [80]. In EMT-6 mammary fibrosarcoma model, rechallenge studies also revealed retardation of secondary tumors inoculated after initial cure [78].

Clinical studies revealed that in patients responding to PDT, there is a significant post-treatment tumor infiltration with CD8⁺ T cells. Decreased expression of MHC class I molecules due to infection with human papillomaviruses (HPV) was associated with poorer responses [81]. It should also be emphasized that at least in one experimental model PDT was observed to decrease MHC class I expression on tumor cells [82], thereby rendering tumor cells insensitive to specific lysis mediated by T cells. There are also studies that indicate lack of systemic immune response induced by local PDT. In a rat model of colon carcinoma growing in the liver, PDT was effective in causing necrosis of illuminated tumors but it did not affect the growth of neighboring, non-illuminated tumors [83]. In another study, PDT was even associated with accelerated progression of non-illuminated metastases [84]. It would be of great translational significance if we could identify factors that determine induction of concomitant immunity after PDT in some tumor models or its absence in other.

Altogether, most of the observations indicate that adaptive immunity is effectively participating

in antitumor effectiveness of PDT. Although it seems that specific immune responses are not particularly effective in the initial tumor ablation, they might contribute to long-term control over the tumor cells. It can be hypothesized at the moment that optimal antitumor treatment involving PDT will include combinations with immunotherapeutic approaches that will facilitate development of concomitant immunity.

Combination of PDT with immunotherapy

PDT has been combined with a number of non-specific immunostimulatory substances. Effective potentiation of the antitumor effects of PDT have been observed after administration of recombinant cytokines (G-CSF, GM-CSF, TNF, TRAIL, FasL, IL-7), bacterial vaccines based on *Corynebacterium parvum*, BCG or bacterial and synthetic immunostimulants (endotoxin, glycated chitosan, schizophyllan, OK-432 and mycobacterial cell wall extract) [35,38,39,54,85–90]. Unexpectedly, we have not observed any potentiation of antitumor effects of PDT by IL-12, IL-18 or bacterial CpG in Balb/C mice inoculated with C-26 cells and treated with Photofrin-based PDT (our unpublished results), which have previously been shown to induce strong antitumor effects [38,91–93].

Potentiated antitumor effects against IL-6 expressing tumors suggest that this cytokine can somehow sensitize tumor cells to PDT [94]. However, other studies have shown that PDT-treated tumor cells become unresponsive to IL-6 [95] or that this cytokine protects tumor cells from PDT-induced apoptosis [96]. These observations cast doubt on the potential application of IL-6 in combined photodynamic immunotherapy.

An interesting approach to induce an antitumor immunity involved selective photodestruction of suppressor T cells that were targeted by conjugates of a photosensitizer and a monoclonal antibody [97]. The antitumor effectiveness of PDT has also been potentiated by adoptively transferred NK cells engineered to produce IL-2 [98].

Phototargeting PDT

The phenomenon of PDT is based on the limited activation of cytotoxic prodrug only in the tumor and surrounding area after systemic injection of photosensitizer via delivery of light. As already mentioned, photosensitizing agents tend to accumulate preferentially in the tumor as compared to normal tissue, although the mechanisms of this phenomenon are not well understood. New

approach called photoimmunotherapy (PIT) may become an interesting alternative to further increase concentration of photosensitizer in the tumor while sparing normal tissue and thus, reducing phototoxicity of PDT. This treatment strategy combines selectivity of monoclonal antibodies or other targeting molecules such as growth factors or peptides binding to growth factor receptors with cytotoxic properties of photosensitizer [99,100]. An antibody is coupled to photosensitizer in such way that biological properties of antibody as well as photophysical activity of photosensitizer are preserved. Although the first report employing such strategy has been published in 1983 [101], PIT has expanded only recently with the advent of monoclonal antibodies in cancer treatment and discovery of several tumor-specific antigens. Numerous antigens present on the surface of tumor cells have been described and may serve as a potential target for PIT. Such approach may be especially effective in tumors, which are not well localized, thus limiting classical PDT and may allow delivery of PDT to large areas [102]. Additionally, photosensitizers can be coupled to such targeting molecules that either remain attached to plasma membrane receptors or become internalized and routed through endocytic pathways to lysosomes where they may be released and redistributed to various cellular compartments.

One of the major problems in the development of PIT is the choice of the photosensitizer. Especially important is its hydrophilic or hydrophobic character. Hydrophobic photosensitizers, such as *meta*-tetrahydroxyphenylchlorin (*m*THPC) penetrate well to the cell after binding of immunconjugate to target cell [103]. However, poor solubility in water hampers their bioavailability and further development. On the other hand, hydrophilic photosensitizers would be much better partners for antibodies and would have better pharmacokinetic properties, but they are not able to enter target cell through phospholipid cell membrane. This classical dilemma of being caught between Scylla and Charybdis may be solved by coupling of hydrophilic photosensitizer to internalizing antibodies. It has been shown that hydrophilic photosensitizer coupled to internalizing antibody presented 1000-fold increase in toxicity in comparison to its free form [104].

Photoimmunotherapy awaits further evaluation and despite significant advances in specific delivery of photosensitizer, still remains an experimental approach. However, successful introduction of several monoclonal antibodies and radioimmunconjugates to clinical protocols in recent years warrants extensive studies on PIT in near future.

Immunoregulatory effects of PDT

The influence of PDT on the immune response is enormously complex. PDT can either stimulate or suppress immune reactivity. Which effect is dominant depends on many factors that include but are seemingly not limited to factors such as particular photosensitizer, fluence, fluence rate, wavelength (tissue penetration) and the total surface area exposed to the treatment. Depending on these variables, PDT can alter the balance between stimulation or regulation of the immune response by the release of certain cytokines, expression of heat shock proteins or the release of antigens from the treated tissue. In this context, it should be stressed that although PDT seems to make tumor cells more immunogenic its direct effect on immune cells is generally harmful.

The first observation that PDT can be immunosuppressive came from the animal studies. It was noticed that skin exposure to the light after a photosensitizer administration resulted in the systemic immunosuppression manifested by the inhibition of contact hypersensitivity (CHS) response [105]. Similar observations were made using different photosensitizers [106,107]. Exposure of skin sections to PDT in vitro prolonged the survival of allografts [108]. The same results were observed following the pretreatment of recipient mice with PDT. In the study mentioned, in PDT-treated mice peritoneal lymphocytes were nearly completely depleted and unresponsive to different mitogens [109]. PDT of the peritoneal cavity caused prolonged systemic immunosuppression [110], mediated by macrophages [111].

Direct influence of PDT on immune cells

There is evidence that immune cells can accumulate diverse photosensitizers and undergo photodynamic reaction [112]. Resting lymphocytes exposed to 5-aminolevulinic acid (ALA) do not accumulate protoporphyrin IX (PpIX). When activated, lymphocytes not only respond to ALA treatment by accumulation of PpIX but also die due to the photodynamic therapy [112]. Similar results were obtained using different photosensitizers [113]. In resting T-cells exposed to PDT only a slight decrease in the expression of MHC class I molecule was observed [113], while in the activated ones lower CD25 levels and a temporary block in cell cycle transition were found [113]. Antigen-presenting cells such as dendritic cells and macrophages, even resting, immediately accumulate ALA and exposed to the light have decreased ability to activate lymphocytes [112]. PDT reduces

the expression of HLA-DR antigens (MHC class II molecules) on human peripheral mononuclear cells [114] and decreases the ATPase activity of murine epidermal Langerhans cells [115]. PDT alters surface receptors of the murine splenic dendritic cells. Decreased expression of MHC class I and II antigens, intercellular adhesion molecule-1 (ICAM-1 and CD54), the co-stimulatory B7-1 (CD80) and B7-2 (CD86) molecules, leukocyte common antigen CD45 and Fas receptor (CD95) as well as integrin CD11c was observed [116]. All these changes may contribute to the immunomodulatory effectiveness of PDT.

Macrophages acquire photosensitizers predominantly by phagocytosis [117]. It was shown that systemic immunosuppression induced by photodynamic therapy is adoptively transferred to the naïve recipients by these cells [111]. PDT inhibits the high-affinity Fc receptor (Fc γ RI) on human monocytes, probably due to the generation of superoxide radicals [118]. The inhibition is caused by a structural alteration of the receptor rather than the loss of the molecule from the cell surface [118]. Fc γ RI takes part in the antibody-dependent cellular cytotoxicity and immunophagocytosis, both crucial events in the efficient immunological response. IFN- γ -activated macrophages are more prone to PDT-induced damage than the resting ones, while treatment of macrophages with LPS makes them resistant to the cytotoxic effects of PDT [119].

Systemic immunoregulation by PDT

The site of irradiation as well as its area are crucial for the immunosuppressive effects of PDT. In a murine model, shielding of the internal organs from PDT did not cause the suppression of CHS, suggesting that internal organs rather than skin are the source of the immunosuppressive agents [120]. PDT induces production of IL-10 in the skin [121]. IL-10 is an anti-inflammatory cytokine that predominantly suppresses cell-mediated immune response [122]. This observation suggests that IL-10 may be responsible for the immunosuppressive effects of PDT. Mice lacking IL-10 expression were found resistant to PDT-mediated inhibition of CHS and treatment of normal mice with anti-IL-10 antibody as well as with recombinant IL-12 reversed the immunosuppressive properties of PDT [123]. The activation of the IL-10 gene promoter in murine keratinocytes exposed to PDT has also been described [124]. Nevertheless, there is a contrasting observations that IL-10 may not be responsible for the PDT-induced suppression of the contact hypersensitivity response [125].

Therapeutic implications of PDT-mediated immunoregulation

The use of PDT in the treatment of autoimmune diseases

All the observations mentioned above have led scientists to the use of PDT in the treatment of some animal models of the autoimmune diseases, e.g. adjuvant-enhanced arthritis and experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). PDT treatment in mice delayed onset, reduced incidence as well as severity of arthritis. PDT also prevented the inflammatory damage to cartilage and bone tissues. The effect was probably due to the selective destruction of adjuvant-activated lymphocytes in the bloodstream as well as in the joints [126]. PDT can be used for the synovectomy with no risk of damage to non-pathologic tissues, which is an advantage comparing with traditional synovectomy techniques [127]. In a murine model of EAE, transcutaneous PDT using verteporfin as a photosensitizer and whole-body light exposure attenuated symptoms and delayed the onset of this disease [128]. Extracorporeal photochemotherapy with 8-methoxypsoralen (8-MOP) and ultraviolet A (UVA) irradiation of the suspension of mononuclear cells in EAE-bearing rats resulted in the reduction of EAE severity, possibly due to the downregulation of the activity of the T-helper 1 (Th1) lymphocytes [129]. The same method was used in the treatment of five patients suffering from the secondary progressive form of MS. Unfortunately, only transient curative effect was observed, suggesting that further studies for this treatment method in humans are needed [130]. Immunosuppressive effects of PDT are used in the treatment of psoriasis, a dermatological disease with a strong autoimmune background. ALA-mediated PDT induced apoptosis of T lymphocytes infiltrating the psoriatic plaques, suggesting a promising role for this treatment in the management of psoriasis [131]. Furthermore, PDT inhibited the secretion of some proinflammatory cytokines (e.g. IL-1 β , TNF and IL-6) by peripheral mononuclear cells, but with a lower effectiveness than PUVA—a standard method of the treatment of psoriasis [132]. Unfortunately, recent results of randomized, observer-blinded studies of topical ALA-based PDT in the management of psoriasis revealed unsatisfactory clinical response and frequent occurrence of pain suggesting that this method is inadequate for the treatment of psoriasis [108].

Although there is a strong immunosuppressive potential of PDT, the use of this therapeutic method in humans needs further studies of its effectiveness, safety and mechanisms of action involved.

The use of PDT in the treatment of graft-versus-host disease

Graft-versus-host disease (GVHD) is a serious complication of allogeneic hematopoietic stem cells transplantation. In immunocompromised host, it may be fatal and is a major obstacle in preventing higher curative rate of allogeneic bone marrow transplantation. Therefore, development of an alternative treatment to suppress this unwanted effect becomes a priority in hematological transplantation, especially in the severe, steroid-refractory form of the disease [133].

Current approaches with PDT as an alternative therapeutic modality in severe GVHD comprise extracorporeal photochemotherapy (ECP) and photodynamic purging. The first approach requires isolation of patients' blood mononuclear cells by apheresis, subsequent photosensitization with 8-MOP and irradiation with UVA. Similar treatment has been used with a limited success in cutaneous T-cell lymphoma (mycosis fungoides) and selected autoimmune diseases [134,135]. ECP has shown some efficacy in the treatment of steroid-refractory chronic GVHD in adults and also in acute pediatric GVHD [136,137]. There are several unresolved issues. One is the timing of ECP. Current data suggest that the treatment should be applied before the onset of the disease.

A positive aspect of GVHD that validates the use of bone marrow transplantation as a treatment of choice in a number of hematological malignancies is a phenomenon called graft versus leukemia. This effect is mediated by alloreactive T cells that act as tumor-specific effectors not involved in GVHD reaction. Selective elimination of the alloreactive population of lymphocytes responsible for GVHD and not for GVL would be desirable. Promising results have been observed in preclinical studies with photoactive rhodamine derivative, which requires visible light for activation and is selectively retained in mitochondria of activated cells. Activated alloreactive T cells were selectively depleted without hampering other T cells [138]. To translate these results into humans, donor leukocytes need to be stimulated exclusively by host T cells in order to avoid depletion of all beneficial anti-leukemic T cells [139].

PDT can also be used in a process referred to as photodynamic cell purging process. This strategy has been already applied to purify autologous bone marrow from malignant cells in several hematological malignancies, including chronic myelogenous leukemia, non-Hodgkin lymphoma and chronic lymphocytic leukemia [140,141] and solid tumors including breast cancer [142,143].

Summary

Photodynamic therapy has become a focus of intense research in the last decade. Multiple mechanisms of its molecular mechanisms have been identified in animal models. Experimental studies revealed that curative PDT directly kills not more than 1–2 logs of tumor cells, far too less than 8-log reduction required for tumor cure [144,145]. We are now aware that PDT leads to both direct as well as indirect antitumor effects. The latter include destruction of blood vessels and activation of early inflammatory response followed by the development of effective concomitant immunity. In contrast to chemotherapy and radiotherapy, which in their current use are inherently immunosuppressive, PDT offers a remarkable advantage of stimulating an immune response. These powerful mechanisms have not yet been effectively translated into clinical practice. The time is now to exploit the potency of the immune system in more effective therapeutic regimens of PDT.

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References

- [1] Castano AP, Demidova TN, Hamblin MR. Mechanisms in photodynamic therapy: part one—photosensitizers, photochemistry and cellular localization. *Photodiagn Photodyn Ther* 2004;1:279–93.
- [2] Castano AP, Demidova TN, Hamblin MR. Mechanisms in photodynamic therapy: part two—cellular signaling, cell metabolism and modes of cell death. *Photodiagn Photodyn Ther* 2005;2:1–23.
- [3] Castano AP, Demidova TN, Hamblin MR. Mechanisms in photodynamic therapy: part three—photosensitizer pharmacokinetics, biodistribution, tumor localization and modes of tumor destruction. *Photodiagn Photodyn Ther* 2005;2:91–106.
- [4] Nowis D, Makowski M, Stoklosa T, Legat M, Issat T, Golab J. Direct tumor damage mechanisms of photodynamic therapy. *Acta Biochim Pol* 2005;52:339–52.
- [5] Agostinis P, Buytaert E, Breyssens H, Hendrickx N. Regulatory pathways in photodynamic therapy induced apoptosis. *Photochem Photobiol Sci* 2004;3:721–9.
- [6] Plaetzer K, Kiesslich T, Oberdanner CB, Krammer B. Apoptosis following photodynamic tumor therapy: induction, mechanisms and detection. *Curr Pharm Des* 2005;11:1151–65.
- [7] Fingar VH, Taber SW, Haydon PS, Harrison LT, Kempf SJ, Wieman TJ. Vascular damage after photodynamic therapy of solid tumors: a view and comparison of effect in pre-

- clinical and clinical models at the University of Louisville. *In Vivo* 2000;14:93–100.
- [8] Krammer B. Vascular effects of photodynamic therapy. *Anticancer Res* 2001;21:4271–7.
- [9] Gilissen MJ, van de Merbel-de Wit LE, Star WM, Koster JF, Sluiter W. Effect of photodynamic therapy on the endothelium-dependent relaxation of isolated rat aortas. *Cancer Res* 1993;53:2548–52.
- [10] Riedemann NC, Ward PA. Complement in ischemia reperfusion injury. *Am J Pathol* 2003;162:363–7.
- [11] Korbelik M, Sun J, Zeng H. Ischaemia-reperfusion injury in photodynamic therapy-treated mouse tumours. *Br J Cancer* 2003;88:760–6.
- [12] Curnow A, Bown SG. The role of reperfusion injury in photodynamic therapy with 5-aminolaevulinic acid—a study on normal rat colon. *Br J Cancer* 2002;86:989–92.
- [13] Korbelik M, Parkins CS, Shibuya H, Cecic I, Stratford MR, Chaplin DJ. Nitric oxide production by tumour tissue: impact on the response to photodynamic therapy. *Br J Cancer* 2000;82:1835–43.
- [14] Golab J, Nowis D, Skrzycki M, et al. Antitumor effects of photodynamic therapy are potentiated by 2-methoxyestradiol. A superoxide dismutase inhibitor. *J Biol Chem* 2003;278:407–14.
- [15] Cecic I, Korbelik M. Mediators of peripheral blood neutrophilia induced by photodynamic therapy of solid tumors. *Cancer Lett* 2002;183:43–51.
- [16] Cecic I, Parkins CS, Korbelik M. Induction of systemic neutrophil response in mice by photodynamic therapy of solid tumors. *Photochem Photobiol* 2001;74:712–20.
- [17] Del Conde I, Cruz MA, Zhang H, Lopez JA, Afshar-Kharghan V. Platelet activation leads to activation and propagation of the complement system. *J Exp Med* 2005;201:871–9.
- [18] Korbelik M, Sun J, Cecic I, Serrano K. Adjuvant treatment for complement activation increases the effectiveness of photodynamic therapy of solid tumors. *Photochem Photobiol Sci* 2004;3:812–6.
- [19] Matroule JY, Bonizzi G, Morliere P, et al. Porphyrin-mediated photo-sensitization activates transcription factor NF-kappaB through the interleukin-1 receptor-dependent signaling pathway. *J Biol Chem* 1999;274:2988–3000.
- [20] Kick G, Messer G, Goetz A, Plewig G, Kind P. Photodynamic therapy induces expression of interleukin 6 by activation of AP-1 but not NF-kappa B DNA binding. *Cancer Res* 1995;55:2373–9.
- [21] Granville DJ, Carthy CM, Jiang H, et al. Nuclear factor-kappaB activation by the photochemotherapeutic agent verteporfin. *Blood* 2000;95:256–62.
- [22] Korbelik M. Induction of tumor immunity by photodynamic therapy. *J Clin Laser Med Surg* 1996;14:329–34.
- [23] Dellian M, Abels C, Kuhnle GE, Goetz AE. Effects of photodynamic therapy on leucocyte–endothelium interaction: differences between normal and tumour tissue. *Br J Cancer* 1995;72:1125–30.
- [24] de Vree WJ, Essers MC, Koster JF, Sluiter W. Role of interleukin 1 and granulocyte colony-stimulating factor in photofrin-based photodynamic therapy of rat rhabdomyosarcoma tumors. *Cancer Res* 1997;57:2555–8.
- [25] Ferrario A, Gomer CJ. Systemic toxicity in mice induced by localized porphyrin photodynamic therapy. *Cancer Res* 1990;50:539–43.
- [26] Canter RJ, Mick R, Kesmodel SB, et al. Intraperitoneal photodynamic therapy causes a capillary-leak syndrome. *Ann Surg Oncol* 2003;10:514–24.
- [27] Yom SS, Busch TM, Friedberg JS, et al. Elevated serum cytokine levels in mesothelioma patients who have undergone pleurectomy or extrapleural pneumonectomy and adjuvant intraoperative photodynamic therapy. *Photochem Photobiol* 2003;78:75–81.
- [28] Fingar VH, Wieman TJ, Wiehle SA, Cerrito PB. The role of microvascular damage in photodynamic therapy: the effect of treatment on vessel constriction, permeability, and leukocyte adhesion. *Cancer Res* 1992;52:4914–21.
- [29] Sun J, Cecic I, Parkins CS, Korbelik M. Neutrophils as inflammatory and immune effectors in photodynamic therapy-treated mouse SCCVII tumours. *Photochem Photobiol Sci* 2002;1:690–5.
- [30] Gollnick SO, Evans SS, Baumann H, et al. Role of cytokines in photodynamic therapy-induced local and systemic inflammation. *Br J Cancer* 2003;88:1772–9.
- [31] Kobayashi W, Liu Q, Matsumiya T, et al. Photodynamic therapy upregulates expression of Mac-1 and generation of leukotriene B(4) by human polymorphonuclear leukocytes. *Oral Oncol* 2004;40:506–10.
- [32] van Duijnhoven FH, Aalbers RI, Rovers JP, Terpstra OT, Kuppen PJ. The immunological consequences of photodynamic treatment of cancer, a literature review. *Immunobiology* 2003;207:105–13.
- [33] Peng Q, Nesland JM. Effects of photodynamic therapy on tumor stroma. *Ultrastruct Pathol* 2004;28:333–40.
- [34] Korbelik M, Cecic I. Contribution of myeloid and lymphoid host cells to the curative outcome of mouse sarcoma treatment by photodynamic therapy. *Cancer Lett* 1999;137:91–8.
- [35] de Vree WJ, Essers MC, de Bruijn HS, Star WM, Koster JF, Sluiter W. Evidence for an important role of neutrophils in the efficacy of photodynamic therapy in vivo. *Cancer Res* 1996;56:2908–11.
- [36] Lim HW, Young L, Hagan M, Gigli I. Delayed phase of hematoporphyrin-induced phototoxicity: modulation by complement, leukocytes, and antihistamines. *J Invest Dermatol* 1985;84:114–7.
- [37] Fingar VH, Siegel KA, Wieman TJ, Doak KW. The effects of thromboxane inhibitors on the microvascular and tumor response to photodynamic therapy. *Photochem Photobiol* 1993;58:393–9.
- [38] Golab J, Wilczynski G, Zagozdzon R, et al. Potentiation of the anti-tumour effects of Photofrin-based photodynamic therapy by localized treatment with G-CSF. *Br J Cancer* 2000;82:1485–91.
- [39] Kros J, Korbelik M, Kros J, Dougherty GJ. Potentiation of photodynamic therapy-elicited antitumor response by localized treatment with granulocyte-macrophage colony-stimulating factor. *Cancer Res* 1996;56:3281–6.
- [40] De Vree WJ, de Bruijn HS, Kraak-Slee G, Koster JF, Sluiter W. The effect of thrombocytopenia on the efficacy of Photofrin-based photodynamic therapy in vivo. *Proc SPIE-Int Soc Opt Eng* 2001;4146:79–85.
- [41] Henderson BW, Gollnick SO, Snyder JW, et al. Choice of oxygen-conserving treatment regimen determines the inflammatory response and outcome of photodynamic therapy of tumors. *Cancer Res* 2004;64:2120–6.
- [42] Miyasaka M, Tanaka T. Lymphocyte trafficking across high endothelial venules: dogmas and enigmas. *Nat Rev Immunol* 2004;4:360–70.
- [43] Reiter I, Schwamberger G, Krammer B. Effect of photodynamic pretreatment on the susceptibility of murine tumor cells to macrophage antitumor mechanisms. *Photochem Photobiol* 1997;66:384–8.
- [44] Evans S, Matthews W, Perry R, Fraker D, Norton J, Pass HI. Effect of photodynamic therapy on tumor necrosis factor production by murine macrophages. *J Natl Cancer Inst* 1990;82:34–9.

- [45] Yamamoto N, Homma S, Sery TW, Donoso LA, Hooper JK. Photodynamic immunopotential: in vitro activation of macrophages by treatment of mouse peritoneal cells with haematoporphyrin derivative and light. *Eur J Cancer* 1991;27:467–71.
- [46] Yamamoto N, Hooper JK, Yamamoto N, Yamamoto S. Tumoricidal capacities of macrophages photodynamically activated with hematoporphyrin derivative. *Photochem Photobiol* 1992;56:245–50.
- [47] Coutier S, Bezdetnaya L, Marchal S, et al. Foscan (mTHPC) photosensitized macrophage activation: enhancement of phagocytosis, nitric oxide release and tumour necrosis factor- α -mediated cytolytic activity. *Br J Cancer* 1999;81:37–42.
- [48] Kros G, Korbek M, Dougherty GJ. Induction of immune cell infiltration into murine SCCVII tumour by photofrin-based photodynamic therapy. *Br J Cancer* 1995;71:549–55.
- [49] Reiter I, Schwamberger G, Krammer B. Activation of macrophage tumoricidal activity by photodynamic treatment in vitro—indirect activation of macrophages by photodynamically killed tumor cells. *J Photochem Photobiol B* 1999;50:99–107.
- [50] Deininger MH, Weinschenk T, Morgalla MH, Meyermann R, Schluesener HJ. Release of regulators of angiogenesis following Hypocrellin-A and -B photodynamic therapy of human brain tumor cells. *Biochem Biophys Res Commun* 2002;298:520–30.
- [51] Korbek M, Kros G. Enhanced macrophage cytotoxicity against tumor cells treated with photodynamic therapy. *Photochem Photobiol* 1994;60:497–502.
- [52] Zagodzón R, Golab J, Stoklosa T, et al. Effective chemo-immunotherapy of L1210 leukemia in vivo using interleukin-12 combined with doxorubicin but not with cyclophosphamide, paclitaxel or cisplatin. *Int J Cancer* 1998;77:720–7.
- [53] Korbek M, Naraparaju VR, Yamamoto N. Macrophage-directed immunotherapy as adjuvant to photodynamic therapy of cancer. *Br J Cancer* 1997;75:202–7.
- [54] Korbek M, Cecic I. Enhancement of tumour response to photodynamic therapy by adjuvant mycobacterium cell-wall treatment. *J Photochem Photobiol B* 1998;44:151–8.
- [55] Yang L, Carbone DP. Tumor–host immune interactions and dendritic cell dysfunction. *Adv Cancer Res* 2004;92:13–27.
- [56] Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. *Nat Rev Immunol* 2005;5:296–306.
- [57] Pardoll D. Does the immune system see tumors as foreign or self? *Annu Rev Immunol* 2003;21:807–39.
- [58] Fuchs EJ, Matzinger P. Is cancer dangerous to the immune system? *Semin Immunol* 1996;8:271–80.
- [59] Guermonprez P, Valladeau J, Zitvogel L, Thery C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol* 2002;20:621–67.
- [60] O'Neill DW, Adams S, Bhardwaj N. Manipulating dendritic cell biology for the active immunotherapy of cancer. *Blood* 2004;104:2235–46.
- [61] Wells AD, Malkovsky M. Heat shock proteins, tumor immunogenicity and antigen presentation: an integrated view. *Immunol Today* 2000;21:129–32.
- [62] Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoeediting. *Annu Rev Immunol* 2004;22:329–60.
- [63] Mazzoni A, Segal DM. Controlling the Toll road to dendritic cell polarization. *J Leukoc Biol* 2004;75:721–30.
- [64] Cottrez F, Groux H. Specialization in tolerance: innate CD(4+)CD(25+) versus acquired TR1 and TH3 regulatory T cells. *Transplantation* 2004;77:S12–5.
- [65] Jakobisiak M, Lasek W, Golab J. Natural mechanisms protecting against cancer. *Immunol Lett* 2003;90:103–22.
- [66] Smyth MJ, Cretney E, Kershaw MH, Hayakawa Y. Cytokines in cancer immunity and immunotherapy. *Immunol Rev* 2004;202:275–93.
- [67] Frandji P, Tkaczyk C, Oskeritzian C, David B, Desaynard C, Mecheri S. Exogenous and endogenous antigens are differentially presented by mast cells to CD4+ T lymphocytes. *Eur J Immunol* 1996;26:2517–28.
- [68] Stoppacciaro A, Melani C, Parenza M, et al. Regression of an established tumor genetically modified to release granulocyte colony-stimulating factor requires granulocyte-T cell cooperation and T cell-produced interferon gamma. *J Exp Med* 1993;178:151–61.
- [69] Skoberne M, Beignon AS, Larsson M, Bhardwaj N. Apoptotic cells at the crossroads of tolerance and immunity. *Curr Top Microbiol Immunol* 2005;289:259–92.
- [70] Morelli AE, Thomson AW. Dendritic cells: regulators of alloimmunity and opportunities for tolerance induction. *Immunol Rev* 2003;196:125–46.
- [71] van Eden W, van der Zee R, Prakken B. Heat-shock proteins induce T-cell regulation of chronic inflammation. *Nat Rev Immunol* 2005;5:318–30.
- [72] Nicchitta CV. Re-evaluating the role of heat-shock protein–peptide interactions in tumour immunity. *Nat Rev Immunol* 2003;3:427–32.
- [73] Chang MK, Binder CJ, Miller YI, et al. Apoptotic cells with oxidation-specific epitopes are immunogenic and proinflammatory. *J Exp Med* 2004;200:1359–70.
- [74] Prasad SJ, Farrand KJ, Matthews SA, Chang JH, McHugh RS, Ronchese F. Dendritic cells loaded with stressed tumor cells elicit long-lasting protective tumor immunity in mice depleted of CD4+CD25+ regulatory T cells. *J Immunol* 2005;174:90–8.
- [75] Jalili A, Makowski M, Switaj T, et al. Effective photoimmunotherapy of murine colon carcinoma induced by the combination of photodynamic therapy and dendritic cells. *Clin Cancer Res* 2004;10:4498–508.
- [76] Gollnick SO, Vaughan L, Henderson BW. Generation of effective antitumor vaccines using photodynamic therapy. *Cancer Res* 2002;62:1604–8.
- [77] Korbek M, Sun J, Cecic I. Photodynamic therapy-induced cell surface expression and release of heat shock proteins: relevance for tumor response. *Cancer Res* 2005;65:1018–26.
- [78] Hendrzak-Henion JA, Knisely TL, Cincotta L, Cincotta E, Cincotta AH. Role of the immune system in mediating the antitumor effect of benzophenothiazine photodynamic therapy. *Photochem Photobiol* 1999;69:575–81.
- [79] Korbek M, Dougherty GJ. Photodynamic therapy-mediated immune response against subcutaneous mouse tumors. *Cancer Res* 1999;59:1941–6.
- [80] Canti G, Lattuada D, Nicolin A, Taroni P, Valentini G, Cubeddu R. Antitumor immunity induced by photodynamic therapy with aluminum disulfonated phthalocyanines and laser light. *Anticancer Drugs* 1994;5:443–7.
- [81] Abdel-Hady ES, Martin-Hirsch P, Duggan-Keen M, et al. Immunological and viral factors associated with the response of vulval intraepithelial neoplasia to photodynamic therapy. *Cancer Res* 2001;61:192–6.
- [82] Blom DJ, Schuitmaker HJ, de Waard-Siebinga I, Dubbelman TM, Jager MJ. Decreased expression of HLA class I on ocular melanoma cells following in vitro photodynamic therapy. *Cancer Lett* 1997;112:239–43.
- [83] van Duijnhoven FH, Aalbers RI, Rovers JP, Terpstra OT, Kuppen PJ. Immunological aspects of photodynamic therapy

- of liver tumors in a rat model for colorectal cancer. *Photochem Photobiol* 2003;78:235–40.
- [84] Momma T, Hamblin MR, Wu HC, Hasan T. Photodynamic therapy of orthotopic prostate cancer with benzoporphyrin derivative: local control and distant metastasis. *Cancer Res* 1998;58:5425–31.
- [85] Chen WR, Korbek M, Bartels KE, Liu H, Sun J, Nordquist RE. Enhancement of laser cancer treatment by a chitosan-derived immunoadjuvant. *Photochem Photobiol* 2005;81:190–5.
- [86] Kros G, Korbek M. Potentiation of photodynamic therapy by immunotherapy: the effect of schizophyllan (SPG). *Cancer Lett* 1994;84:43–9.
- [87] Uehara M, Sano K, Wang ZL, Sekine J, Ikeda H, Inokuchi T. Enhancement of the photodynamic antitumor effect by streptococcal preparation OK-432 in the mouse carcinoma. *Cancer Immunol Immunother* 2000;49:401–9.
- [88] Myers RC, Lau BH, Kunihira DY, Torrey RR, Woolley JL, Tosk J. Modulation of hematoporphyrin derivative-sensitized phototherapy with *Corynebacterium parvum* in murine transitional cell carcinoma. *Urology* 1989;33:230–5.
- [89] Granville DJ, Jiang H, McManus BM, Hunt DW. Fas ligand and TRAIL augment the effect of photodynamic therapy on the induction of apoptosis in JURKAT cells. *Int Immunopharmacol* 2001;1:1831–40.
- [90] Bellnier DA. Potentiation of photodynamic therapy in mice with recombinant human tumor necrosis factor- α . *J Photochem Photobiol B* 1991;8:203–10.
- [91] Switaj T, Jalili A, Jakubowska AB, et al. CpG immunostimulatory oligodeoxynucleotide 1826 enhances antitumor effect of interleukin 12 gene-modified tumor vaccine in a melanoma model in mice. *Clin Cancer Res* 2004;10:4165–75.
- [92] Kozar K, Kaminski R, Switaj T, et al. Interleukin 12-based immunotherapy improves the antitumor effectiveness of a low-dose 5-Aza-2'-deoxycytidine treatment in L1210 leukemia and B16F10 melanoma models in mice. *Clin Cancer Res* 2003;9:3124–33.
- [93] Golab J, Zagodzón R. Antitumor effects of interleukin-12 in pre-clinical and early clinical studies (Review). *Int J Mol Med* 1999;3:537–44.
- [94] Usuda J, Okunaka T, Furukawa K, et al. Increased cytotoxic effects of photodynamic therapy in IL-6 gene transfected cells via enhanced apoptosis. *Int J Cancer* 2001;93:475–80.
- [95] Liu W, Oseroff AR, Baumann H. Photodynamic therapy causes cross-linking of signal transducer and activator of transcription proteins and attenuation of interleukin-6 cytokine responsiveness in epithelial cells. *Cancer Res* 2004;64:6579–87.
- [96] Jee SH, Shen SC, Chiu HC, Tsai WL, Kuo ML. Overexpression of interleukin-6 in human basal cell carcinoma cell lines increases anti-apoptotic activity and tumorigenic potency. *Oncogene* 2001;20:198–208.
- [97] Steele JK, Liu D, Stammers AT, Whitney S, Levy JG. Suppressor deletion therapy: selective elimination of T suppressor cells in vivo using a hematoporphyrin conjugated monoclonal antibody permits animals to reject syngeneic tumor cells. *Cancer Immunol Immunother* 1988;26:125–31.
- [98] Korbek M, Sun J. Cancer treatment by photodynamic therapy combined with adoptive immunotherapy using genetically altered natural killer cell line. *Int J Cancer* 2001;93:269–74.
- [99] Schneider R, Schmitt F, Frochot C, et al. Design, synthesis, and biological evaluation of folic acid targeted tetraphenylporphyrin as novel photosensitizers for selective photodynamic therapy. *Bioorg Med Chem* 2005;13:2799–808.
- [100] Renno RZ, Terada Y, Haddadin MJ, Michaud NA, Gragoudas ES, Miller JW. Selective photodynamic therapy by targeted verteporfin delivery to experimental choroidal neovascularization mediated by a homing peptide to vascular endothelial growth factor receptor-2. *Arch Ophthalmol* 2004;122:1002–11.
- [101] Mew D, Wat CK, Towers GH, Levy JG. Photoimmunotherapy: treatment of animal tumors with tumor-specific monoclonal antibody-hematoporphyrin conjugates. *J Immunol* 1983;130:1473–7.
- [102] van Dongen GA, Visser GW, Vrouenraets MB. Photosensitizer-antibody conjugates for detection and therapy of cancer. *Adv Drug Deliv Rev* 2004;56:31–52.
- [103] Vrouenraets MB, Visser GW, Stewart FA, et al. Development of meta-tetrahydroxyphenylchlorin-monoclonal antibody conjugates for photoimmunotherapy. *Cancer Res* 1999;59:1505–13.
- [104] Vrouenraets MB, Visser GW, Stigter M, Oppelaar H, Snow GB, van Dongen GA. Targeting of aluminum (III) phthalocyanine tetrasulfonate by use of internalizing monoclonal antibodies: improved efficacy in photodynamic therapy. *Cancer Res* 2001;61:1970–5.
- [105] Elmetts CA, Bowen KD. Immunological suppression in mice treated with hematoporphyrin derivative photoradiation. *Cancer Res* 1986;46:1608–11.
- [106] Simkin GO, King DE, Levy JG, Chan AH, Hunt DW. Inhibition of contact hypersensitivity with different analogs of benzoporphyrin derivative. *Immunopharmacology* 1997;37:221–30.
- [107] Musser DA, Fiel RJ. Cutaneous photosensitizing and immunosuppressive effects of a series of tumor localizing porphyrins. *Photochem Photobiol* 1991;53:119–23.
- [108] Radakovic-Fijan S, Blecha-Thalhammer U, Schleyer V, et al. Topical aminolaevulinic acid-based photodynamic therapy as a treatment option for psoriasis? Results of a randomized, observer-blinded study. *Br J Dermatol* 2005;152:279–83.
- [109] Qin B, Selman SH, Payne KM, Keck RW, Metzger DW. Enhanced skin allograft survival after photodynamic therapy. Association with lymphocyte inactivation and macrophage stimulation. *Transplantation* 1993;56:1481–6.
- [110] Jolles CJ, Ott MJ, Straight RC, Lynch DH. Systemic immunosuppression induced by peritoneal photodynamic therapy. *Am J Obstet Gynecol* 1988;158:1446–53.
- [111] Lynch DH, Haddad S, King VJ, Ott MJ, Straight RC, Jolles CJ. Systemic immunosuppression induced by photodynamic therapy (PDT) is adoptively transferred by macrophages. *Photochem Photobiol* 1989;49:453–8.
- [112] Hryhorenko EA, Oseroff AR, Morgan J, Rittenhouse-Diakun K. Antigen specific and nonspecific modulation of the immune response by aminolaevulinic acid based photodynamic therapy. *Immunopharmacology* 1998;40:231–40.
- [113] Hunt DW, Jiang H, Granville DJ, Chan AH, Leong S, Levy JG. Consequences of the photodynamic treatment of resting and activated peripheral T lymphocytes. *Immunopharmacology* 1999;41:31–44.
- [114] Gruner S, Volk HD, Noack F, Meffert H, von Baehr R. Inhibition of HLA-DR antigen expression and of the allogeneic mixed leukocyte reaction by photochemical treatment. *Tissue Antigens* 1986;27:147–54.
- [115] Gruner S, Meffert H, Volk HD, Grunow R, Jahn S. The influence of haematoporphyrin derivative and visible light on murine skin graft survival, epidermal Langerhans cells and stimulation of the allogeneic mixed leucocyte reaction. *Scand J Immunol* 1985;21:267–73.

- [116] King DE, Jiang H, Simkin GO, Obochi MO, Levy JG, Hunt DW. Photodynamic alteration of the surface receptor expression pattern of murine splenic dendritic cells. *Scand J Immunol* 1999;49:184–92.
- [117] Korbek M, Sun J, Posakony JJ. Interaction between photodynamic therapy and BCG immunotherapy responsible for the reduced recurrence of treated mouse tumors. *Photochem Photobiol* 2001;73:403–9.
- [118] Krutmann J, Athar M, Mendel DB, et al. Inhibition of the high affinity Fc receptor (Fc gamma RI) on human monocytes by porphyrin photosensitization is highly specific and mediated by the generation of superoxide radicals. *J Biol Chem* 1989;264:11407–13.
- [119] Hunt DW, Jiang HJ, Levy JG, Chan AH. Sensitivity of activated murine peritoneal macrophages to photodynamic killing with benzoporphyrin derivative. *Photochem Photobiol* 1995;61:417–21.
- [120] Musser DA, Camacho SH, Manderscheid PA, Oseroff AR. The anatomic site of photodynamic therapy is a determinant for immunosuppression in a murine model. *Photochem Photobiol* 1999;69:222–5.
- [121] Gollnick SO, Liu X, Owczarczak B, Musser DA, Henderson BW. Altered expression of interleukin 6 and interleukin 10 as a result of photodynamic therapy in vivo. *Cancer Res* 1997;57:3904–9.
- [122] Vicari AP, Trinchieri G. Interleukin-10 in viral diseases and cancer: exiting the labyrinth? *Immunol Rev* 2004;202:223–36.
- [123] Simkin GO, Tao JS, Levy JG, Hunt DW. IL-10 contributes to the inhibition of contact hypersensitivity in mice treated with photodynamic therapy. *J Immunol* 2000;164:2457–62.
- [124] Gollnick SO, Lee BY, Vaughan L, Owczarczak B, Henderson BW. Activation of the IL-10 gene promoter following photodynamic therapy of murine keratinocytes. *Photochem Photobiol* 2001;73:170–7.
- [125] Gollnick SO, Musser DA, Oseroff AR, Vaughan L, Owczarczak B, Henderson BW. IL-10 does not play a role in cutaneous Photofrin photodynamic therapy-induced suppression of the contact hypersensitivity response. *Photochem Photobiol* 2001;74:811–6.
- [126] Chowdhary RK, Ratkay LG, Neyndorff HC, et al. The use of transcutaneous photodynamic therapy in the prevention of adjuvant-enhanced arthritis in MRL/lpr mice. *Clin Immunol Immunopathol* 1994;72:255–63.
- [127] Trauner KB, Gandour-Edwards R, Bamberg M, Shortkroff S, Sledge C, Hasan T. Photodynamic synovectomy using benzoporphyrin derivative in an antigen-induced arthritis model for rheumatoid arthritis. *Photochem Photobiol* 1998;67:133–9.
- [128] Leong S, Chan AH, Levy JG, Hunt DW. Transcutaneous photodynamic therapy alters the development of an adoptively transferred form of murine experimental autoimmune encephalomyelitis. *Photochem Photobiol* 1996;64:751–7.
- [129] Cavaletti G, Perseghin P, Dassi M, et al. Extracorporeal photochemotherapy reduces the severity of Lewis rat experimental allergic encephalomyelitis through a modulation of the function of peripheral blood mononuclear cells. *J Biol Regul Homeost Agents* 2004;18:9–17.
- [130] Besnier DP, Chabannes D, Mussini JM, Dupas B, Esnault VL. Extracorporeal photochemotherapy for secondary chronic progressive multiple sclerosis: a pilot study. *Photodermatol Photoimmunol Photomed* 2002;18:36–41.
- [131] Bissonnette R, Tremblay JF, Juzenas P, Boushira M, Lui H. Systemic photodynamic therapy with aminolevulinic acid induces apoptosis in lesional T lymphocytes of psoriatic plaques. *J Invest Dermatol* 2002;119:77–83.
- [132] Boehncke WH, König K, Kaufmann R, Scheffold W, Prummer O, Sterry W. Photodynamic therapy in psoriasis: suppression of cytokine production in vitro and recording of fluorescence modification during treatment in vivo. *Arch Dermatol Res* 1994;286:300–3.
- [133] Svennilson J. Novel approaches in GVHD therapy. *Bone Marrow Transplant* 2005;35(Suppl. 1):S65–7.
- [134] Edstrom DW, Porwit A, Ros AM. Photodynamic therapy with topical 5-aminolevulinic acid for mycosis fungoides: clinical and histological response. *Acta Derm Venereol* 2001;81:184–8.
- [135] Demidova TN, Hamblin MR. Macrophage-targeted photodynamic therapy. *Int J Immunopathol Pharmacol* 2004;17:117–26.
- [136] Greinix HT, Volc-Platzer B, Kalhs P, et al. Extracorporeal photochemotherapy in the treatment of severe steroid-refractory acute graft-versus-host disease: a pilot study. *Blood* 2000;96:2426–31.
- [137] Kanold J, Messina C, Halle P, et al. Update on extracorporeal photochemotherapy for graft-versus-host disease treatment. *Bone Marrow Transplant* 2005;35(Suppl. 1):S69–71.
- [138] Chen BJ, Cui X, Liu C, Chao NJ. Prevention of graft-versus-host disease while preserving graft-versus-leukemia effect after selective depletion of host-reactive T cells by photodynamic cell purging process. *Blood* 2002;99:3083–8.
- [139] Gudgin Dickson EF, Goyan RL, Pottier RH. New directions in photodynamic therapy. *Cell Mol Biol (Noisy-le-grand)* 2002;48:939–54.
- [140] Mulrone CM, Gluck S, Ho AD. The use of photodynamic therapy in bone marrow purging. *Semin Oncol* 1994;21:24–7.
- [141] Villeneuve L. Ex vivo photodynamic purging in chronic myelogenous leukaemia and other neoplasias with rhodamine derivatives. *Biotechnol Appl Biochem* 1999;30(Pt 1):1–17.
- [142] Brasseur N, Menard I, Forget A, et al. Eradication of multiple myeloma and breast cancer cells by TH9402-mediated photodynamic therapy: implication for clinical ex vivo purging of autologous stem cell transplants. *Photochem Photobiol* 2000;72:780–7.
- [143] Anderson GS, Tsujino I, Miyagi K, Sampson R, Sieber F. Preferential inactivation of paediatric solid tumour cells by sequential exposure to Merocyanine 540-mediated photodynamic therapy and Edelfosine: implications for the ex vivo purging of autologous haematopoietic stem cell grafts. *J Photochem Photobiol B* 2003;69:87–95.
- [144] Henderson BW, Waldow SM, Mang TS, Potter WR, Malone PB, Dougherty TJ. Tumor destruction and kinetics of tumor cell death in two experimental mouse tumors following photodynamic therapy. *Cancer Res* 1985;45:572–6.
- [145] Chan WS, Brasseur N, La Madeleine C, van Lier JE. Evidence for different mechanisms of EMT-6 tumor necrosis by photodynamic therapy with disulfonated aluminum phthalocyanine or photofrin: tumor cell survival and blood flow. *Anticancer Res* 1996;16:1887–92.