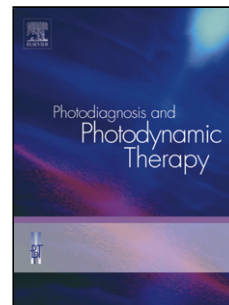


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Immunological effects of photodynamic therapy in the treatment of actinic keratosis and squamous cell carcinoma

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Highlights

- Besides UV-induced DNA damage, UV-induced immunosuppression and inflammation are also essential in the development of actinic keratosis.
- PDT does not only destroy tumor via direct cell destruction and indirectly via vascular shutdown but induce acute local inflammatory response and activates the innate and adaptive immune system.
- Defective immune response to dysplastic keratinocytes may be the target of photodynamic therapy to effectively eliminate actinic keratosis.

ABBREVIATIONS

5-ALA 5 aminolevulinic acid

AK actinic keratosis

APC antigen presenting cell

CHS contact hypersensitivity

CRT calreticulin

cSCC cutaneous squamous cell cancer

DAMP damage-associated molecular pattern

DC dendritic cell

HMGB1 high-mobility group box 1 protein

HPV human papilloma virus

HSP heat shock protein

ICD immunogenic cell death

IEC intraepithelial carcinoma
IL interleukin
LC Langerhans cell
MAC membrane attack complex
MAL methylaminolevulinic acid
MC mast cell
MHC major histocompatibility complex
MMP matrix metalloproteinase
NK cell natural killer cell
UV ultraviolet
PAF platelet-activating factor
PGE₂ prostaglandin E₂
ROS reactive oxygen species
SCC squamous cell carcinoma
TGF- β transforming growth factor β
TLR Toll-like receptor
TNF- α tumor necrosis factor α
Tregs regulatory T cells

Keywords: immunology, photodynamic therapy, actinic keratosis

Word count: 3574

Abstract

The use of photodynamic therapy is extensive, due to its antitumoral, antibacterial and photorejuvenation effects. It destroys tumor via direct cell destruction and indirectly via vascular shutdown, induction of acute local inflammatory response and activation of the immune system. Both innate and adaptive immune cells are involved in the immunological effects of photodynamic therapy. In addition to UV-induced DNA damage, inflammation and immunosuppression are also essential elements in the pathogenesis of actinic keratosis. Both immunosuppression induced by UV and defective immune response to dysplastic keratinocytes may be the target of photodynamic therapy to eliminate actinic keratosis. These elements are discussed in the present review, highlighting the possible mechanism of photodynamic therapy to effectively treat actinic keratosis.

Introduction

Chronic UV exposure has a central role in the pathogenesis of cutaneous squamous cell cancer (cSCC) and its premalignant stage actinic keratosis (AK), through a multi-stage carcinogenesis process. In response to UV exposure, the tumor suppressor gene p53 is expressed and activated in the epidermis to induce cell cycle arrest and activate DNA repair machinery. The mutation of p53 is an early step in tumorigenesis, and characteristic of AK. Further mutations in oncogenes and immunosuppressive genes lead to the development of intraepidermal cancer (IEC) and finally to cSCC. The presence of multiple AK in one region reflects field cancerization, which means that surrounding keratinocytes contain also a certain amount of DNA damage.^{1,2,3} In addition to UV-induced DNA damage, UV-induced inflammation and immunosuppression have major importance in the pathogenesis of AK and cSCC.^{1,2} When UV-induced keratinocyte skin cancer is transplanted into immunocompetent mouse, the tumor regresses, but if the recipient mouse is exposed to UV before transplantation, the tumor survives and grows.⁴ The decreased immune surveillance and tumor growth are due to an impaired antigen presentation (altered morphology of antigen presenting cells (APCs)) and decreased expression of MHC class II, CD40, CD80, CD86 on their surface) and activation of regulatory T cells (Tregs), which are caused by UV-induced immunosuppressive soluble mediators (pl. IL-4, IL-10, TNF- α , PGE₂), reactive oxygen species (ROS), platelet-activating factor (PAF) secretion, isomerization of trans-urocanic acid to the immunosuppressive cis-urocanic acid, Toll-like receptor (TLR) 3 and TLR4 activation.^{5,6} Nevertheless, incidence of AK in immunosuppressed patients is highly increased compared to immunocompetent individuals suggesting that functional immune surveillance is important in the clearance of pre-malignant skin lesions.⁶

The mechanism of action of photodynamic therapy (PDT) is based on the production of ROS, vascular shut down and inflammatory, immunological processes.⁷ These immunological changes can boost anticancer processes but can be immunosuppressive as well.⁸ PDT eventuate a fast-therapeutic effect with excellent cosmetic outcome. However, there is great individual difference in the duration of response after PDT, the reasons for which are unclear.⁹ Although there may be individual differences in the cytotoxic effect (e.g. due to differences in photosensitizer uptake and conversion to PPIX, depth of cells), the induced immune response is likely to contribute to the variation in efficacy. The outcome of PDT, such as therapy resistance, early relapse or long-term remission, is presumably influenced by the relationship between the PDT-induced innate and adaptive immune response and the induced inflammation and immunosuppression in the microenvironment of the lesion. This review intends to address these processes and points out the unexplored issues of this field.

Because squamous cell cancers arise via a neoplastic progression (AK to IEC to SCC), the goal of many studies is to analyze immunological events in each of these stages and compare them to each other. In this review, we summarize the reported data in each of these clinical entities, as well as describe the influence of PDT on individual immune cell populations.

The current knowledge on immune profile of actinic keratosis and squamous cell cancer

The contribution of chronic inflammation to the development of AK and cSCC was mainly investigated on HPV16 mice models.^{10, 11, 12, 13, 14, 15}

There are only a few human studies which analyzed the immune cell composition of these lesions.^{16, 17, 18, 19} Some of them compared the immune profiles in AK and cSCC to the immune infiltrate in photodamaged skin and/or IEC.^{17, 18, 19} The major results are summarized in Table 1.

INNATE IMMUNE CELLS

Langerhans cells and dendritic cells

CD1a⁺ Langerhans cells (LCs) are located in the epidermis, while CD11c⁺ conventional dendritic cells (cDCs) and plasmacytoid DCs are mainly in the dermis. DCs are the link between innate and adaptive immune response. They present antigens via MHC class I and class II complexes.²⁰

Immunohistochemical studies revealed that the presence of LC decreases gradually with malignant transformation.¹⁷ Furthermore, fewer CD11c⁺ HLA-DR⁺ cDCs are present in IEC and cSCC than in photodamaged skin.¹⁹ It might reflect the migration of cDCs from the lesion to the draining lymph node to present tumor associated antigens there.¹⁹ However, it was not confirmed yet with specific investigations. Moreover, Jang found significantly lower number of cDCs in AKs compared to IECs and cSCCs¹⁸, which contradicts to the previous results. In addition, BDCA-2⁺ HLA-DR⁺ plasmacytoid DCs were found to be more abundant in cSCCs compared to photodamaged skin or IEC.¹⁹

NK cells and macrophages

Only one study analyzed the presence of CD3⁻CD56⁺ NK cells, and it has found a higher number of NK cells in photodamaged skin compared to IEC and cSCC.¹⁹

In contrast, the presence of CD68⁺ macrophages was more abundant in cSCCs compared to AKs.^{16, 17}

Neutrophils and mast cells

Both neutrophils and mast cells could be detected in AKs, however, the presence of neutrophils was prominent in dysplastic and carcinoma tissues both in the center and in the border, while mast cells (MCs) were barely seen, in the tumor center.¹⁰ A recent study, using MC-deficient/HPV16 mice model, has found that the absence of MCs does not prevent the development of cSCC and does not alter the immune cell composition in cSCC.¹¹ In contrast, previous publications reported decreased neoplastic progression in MC-deficient/HPV16 mice, which was attributed to weaker activation of angiogenic factors and decreased ability of keratinocytes to reach hyperproliferative status.¹² Furthermore, it has been demonstrated that chronic UV exposure significantly increases the number of MCs in the dermis and the immunosuppressive effect of MCs can promote skin tumor development.¹³ The degree of UV-induced immunosuppression and cSCC prevalence was significantly lower in MC-deficient mice or when cutaneous MC expansion was blocked in murine models.^{12, 14}

Considering the contradictory data, the role of MCs in the development of AK needs to be clarified in the future.

ADAPTIVE IMMUNE CELLS

B cells

It was revealed, that the knock-out of complete adaptive immune system in HPV16 mice leads to the failure of initiation of chronic inflammation in premalignant conditions that results in decreased progression into cancer.¹⁵ When B lymphocytes were transferred to adaptive immune-deficient HPV16 mice, chronic inflammation along with the signs of premalignant transformation could be observed.¹⁵ It suggests the important role of B lymphocytes in the onset of chronic inflammation and premalignant progression. CD20⁺ B cells are components of immune infiltrate in AK in humans, however, these cells seem to be more abundant in cSCCs.¹⁶

T cells

Depending on the local cytokine milieu, which is determined by the APCs, the naïve T cells polarize into effector helper (Th) or cytotoxic T cells, to memory T cells, or to Tregs.²¹ IL-12 secreted by APCs promote the differentiation to Th1 cells. Th1 cells secrete IFN- γ , which contributes to the activation of macrophages, supports activity of NK cells and increase the expression of MHC I and MHC II on APCs and on normal cells. These cells also secrete TNF- α , TNF- β , IL-2 and GM-CSF, which contribute to inflammation via macrophage, DC and complement activation and promote the proliferation of B cells. Th1 cells also play a major role in the activation and proliferation of CD8⁺ T cells²², but it is also supported by IL-17, which is produced by Th17 cells.²³ Th17 cells release IL-17, which promotes the stimulation and generation of neutrophils, but the role of Th17 cells in tumors is ambiguous: depending on the microenvironment, they can act against the cancer or can stimulate tumor progression.²⁴ The presence of low concentrations of transforming growth factor- β (TGF- β) in the microenvironment leads to Th17 polarization, while the presence of high concentrations of TGF- β results in Treg polarization.²⁵ There are two types of regulatory T cells: natural Tregs and induced Tregs (iTregs). Natural Tregs develop in the thymus from CD4⁺ FoxP3⁺ T cells, while iTregs differentiate de novo at the periphery from CD4⁺ CD25⁺ T cells.^{26, 27} Tregs and iTregs have distinct functions, natural Tregs recognize self-antigens, therefore they are important for protection against autoimmunity, while iTregs protect against excessive immune activation in response to non-self-antigens.²⁶ The proliferation of CD4⁺CD25⁺ Tregs is promoted by IL-10 and TGF- β .^{28, 29} Tregs suppress the function of effector T cells via various mechanisms³⁰, and it has been shown that their number is increased in tumor tissues²⁹, even in IEC and in cSCC.¹⁸ They possess immunosuppressive potential by the secretion of IL-10 and TGF- β , leading to the inhibition of CD4⁺ T cell and dendritic cell activation and cytokine production, respectively.¹⁸

In human studies CD3⁺ T cells were found to be present more abundantly in AKs and IEC than in cSCC.^{16, 19} Furthermore, high CD4⁺/CD8⁺ T cell ratio could be detected in cSCCs, suggesting that CD4⁺/CD8⁺ ratio might be a diagnostic indicator for progression into cSCC.¹⁹ In another study, however, the presence of cytotoxic T cells was more abundant in cSCCs than in AK.¹⁶

There was no significant difference in the percentages of FoxP3⁺ Tregs and $\gamma\delta$ T-cells in the photodamaged skin, IEC and cSCC samples.¹⁹ It contradicts the results of Jang, who found higher proportion of FoxP3⁺Tregs in cSCCs than in AKs or IECs.¹⁸ Therefore, the role of Tregs should be also clarified in the future.

Overall, there are only a few studies that analyzed the immune infiltrate of AKs, and these have revealed signs of both immune activation and immunosuppression. Immunosuppressive mechanisms can prevent the elimination of premalignant cells and initiate the development of AK and its progression into IEC and finally to cSCC. PDT can destroy directly the premalignant cells and, as we assume, break through the immunosuppression by inducing immune response to the destroyed cells.³¹

However, more human studies focusing on the contradictory data (e.g. Langerhans cell, mast cell, regulatory T cell) should be performed, to find the answer to the question, is there an immune marker which could indicate treatment efficacy and the optimal therapy to certain patients?

Immunological effects of photodynamic therapy

Photodynamic therapy (PDT) is an effective anticancer treatment modality, which has a long-standing history and a widespread field of application. Basically, three components are needed for PDT: oxygen, photosensitizer and proper wavelength of light. Cells (primarily the rapidly proliferating ones, but tumor stroma cells as well) accumulate the photosensitizer in subcellular organelles, which is then excited by light, resulting in the release of ROS, oxidizing biomolecules, which leads to tissue destruction and elimination of the damaged tissue by apoptosis, necrosis or autophagy.^{32, 33, 34} As an indirect effect, PDT induces vascular shutdown by destroying endothelial cells and the vascular basement membrane, resulting in oxygen deprivation. Moreover, acute local inflammatory and immunological reactions, involving the innate and adaptive immune system, are induced, which contribute not only to control the growth of the primary tumor but also to prevent the development of a second one.^{34, 35} Nevertheless, there are studies suggesting the immunosuppressive effects of ALA-PDT as well.^{36, 37, 38} Hayami et al. used a murine contact hypersensitivity model.³⁶ When trying to induce contact sensitization on previously PDT treated sites, the contact hypersensitivity was suppressed, even on the distant non-PDT treated skin as well.³⁶ Matthews et al. reported human data on the immunosuppressive effect of PDT. They found that both 5-ALA and MAL-PDT could suppress positive Mantoux reaction on healthy volunteers, but only in the treated area.³⁷ Other experiments showed that platelet-activating factor (PAF) and other PAF-receptor ligands are generated locally after PDT, which cause systemic immunosuppression.³⁸ Dose responsiveness of PDT-induced immunosuppression was confirmed in human skin. MAL-PDT at low fluence rate did not lead to immunosuppression, which was evaluated on Mantoux reactions.³⁹

Photosensitizers and DAMPs

There are several types (porphyrins, porphyrin precursors, phthalocyanines, porphycenes, chlorines, pheophorbides, and others like methylene blue, rose Bengal, hypericin) of photosensitizers, which are applied not only for antitumoral but also for antibacterial purposes. They are accumulated in different cell organelles (mitochondria, lysosomes, plasma membrane,

Golgi apparatus, endoplasmic reticulum) and can be administered intravenously, orally or locally. Therefore, PDT is widely used in medicine and is being the subject of extensive research.^{32, 40} 5-aminolevulinic acid (5-ALA) and methylaminolevulinic acid (MAL) have approval in dermatological indications, including actinic keratosis. These two photosensitizers are accumulated in the cytoplasm and mitochondria as well.⁴¹ After illumination with blue, red or sunlight, ROS are generated, which are highly cytotoxic and lead to cell damage.⁴² Damage-associated molecular patterns (DAMPs) are released from dying and injured cells, initiating immune response and antitumor immunity.⁴³ Calreticulin (CRT), heat shock protein (HSP) 70, HSP90, ATP and high-mobility group box 1 protein (HMGB1) are reported to be mainly involved in PDT associated immune response generation, depending on the type of photosensitizer.^{7, 42} ALA-PDT can induce the expression of CRT, HSP70 and HMGB1, which were detected in a murine SCC model.⁴⁴ Among DAMPs, CRT and HMGB1 are thought to be associated with PDT-induced immunogenic cell death (ICD), while HSPs seem to be associated with PDT-induced apoptosis and ICD as well.^{45, 46}

CRT, when appears on the cell surface, serves as a signal for DCs and macrophages to phagocyte the damaged cell and after ingestion the expression of tumor-antigens on APCs along with costimulatory molecules lead to the activation of T-cells.⁴⁷

HSPs, associated with cell membrane not only activate DCs and NK cells but stimulate them to produce pro-inflammatory cytokines (TNF, IL-1 β , IL-12, IL-6) and facilitate the presentation of tumor antigens.^{45, 48, 49, 50, 51, 52} Extracellular HSPs form complexes with tumor-antigens and these complexes are taken up by APCs, initiating adaptive immune response.^{48, 53} Moreover, HSPs can stimulate the migration and maturation of DCs by the upregulation of the expression of MHC class II, CD80, CD86, CD83, and CD40 costimulatory molecules.^{54, 48}

HMGB1 protein regulates the transcription of p53 and nuclear factor- κ B (NF- κ B).⁵⁵ Nevertheless, cell death accompanied by HMGB1 release is a form of ICD and is associated with the production of pro-inflammatory cytokines (TNF- α , IL-1, IL-6, IL-8) by innate immune cells (neutrophils, monocytes and macrophages) which attracts naïve T cells, DCs and monocytes to the site of damage.^{56, 57} The major events induced by PDT are summarized on Figure 1.

Furthermore, extracellular nucleic acids, dsDNA, dsRNA can also act as DAMPs, but are released in later stages of cell death.⁵⁸ They bind to TLR7/8 and TLR9, and activate innate immune cells, including neutrophils and dendritic cells.⁵⁸

Human data and information about the connection between DAMPs and PDT efficacy/inefficacy are missing.

The influence of PDT on immune cells according to the available literature is summarized in Table 2.

What is the role of dendritic cells (DCs) in the effect of PDT?

DCs, as professional antigen presenting cells, recognize DAMPs released by dying/damaged tumor cells. They process antigens and migrate to regional lymph nodes, where they present tumor-associated antigens on their surface by MHCs. As a consequence, CD8⁺ cytotoxic tumor-specific T cells (CTLs) and CD4⁺ T helper cells are primed, and B cells are activated, initiating adaptive immune response.^{20, 36, 46, 59, 60}

More and more information is published regarding PDT's effect on immune cells in mouse SCC models. Wang et al.⁶¹ detected a significant increase in the number of CD1a⁺ DCs 24 hours after ALA-PDT in the treated SCCs. It was confirmed by Ji et al. that ALA-PDT could induce

the maturation of DCs with the upregulation of MHCII, CD80 and CD86 expression and proinflammatory cytokine (IFN- γ and IL-12) secretion.⁶² However, studies on the immunological effect of PDT in non-cancer models showed that PDT could have immunosuppressive effect on skin.^{36, 37} Hayami et al.³⁶ investigated the immunological effects of PDT in murine contact hypersensitivity (CHS) model. The number of Langerhans cells was significantly decreased 1 day after PDT and the remaining ones were rounded and lacked dendrites. LC started to recover 5 days after PDT and reached nearly the same level 2 weeks after the treatment. Meanwhile the number of CD11c⁺ DCs was significantly increased in the draining lymph nodes 24 hours later, up to 5 days after PDT.³⁶ The possible immunosuppressive effect of PDT is also confirmed by human data. Even in healthy human skin, the number of LCs was decreased significantly 24 hours after ALA-PDT.⁶³ Nevertheless, human data are not yet available in the context of immunological effects of PDT on DCs in AK or cSCC.

Are neutrophils involved in the effect of PDT?

PDT induces an acute-phase response, resulting in neutrophil accumulation at the site of the injury. The damage of the vasculature leads to the extravasation of plasma proteins, fragmented extracellular matrix (ECM) and to the release of DAMPs, which recruit neutrophils to the site of the injury.^{64, 65} Furthermore, the complement cascade is also activated, leading to membrane attack complex (MAC) formation with direct damage of tumor and endothelial cells and neutrophil chemotaxis by the presence of C3a and C5a complement degradation products.^{64, 65} Proinflammatory cytokines (IL-1 β , TNF- α , IL-6, IL-8) and arachidonic acid metabolites also attract neutrophils.⁶⁶ Neutrophils can directly kill tumor cells by oxidative burst and ROS production and indirectly by the release of cytokines and chemokines that take part in the recruitment and activation of other immune cells; for example, active neutrophils can facilitate the maturation of DCs.^{57, 66, 67}

Human data are also available proving that neutrophils are involved in PDT mediated immune response. Three hours after 5-ALA PDT of AK, marked neutrophilic infiltrate could be detected both in the epidermis and in the dermis, and their number decreased 3 days after PDT.⁶⁸ Rapid influx of neutrophils was detected in the dermis four hours after PDT in healthy human skin as well but their number decreased to near baseline 24 hours after PDT.⁶³ As LCs undergo phenotypic changes after PDT, it raises the question of whether neutrophils also undergo phenotypic and functional changes after PDT. However, there are no investigations so far to address this question.

What cytokines are released after PDT?

During PDT induced inflammation, various proinflammatory cytokines are released.⁷ The most prominent ones are IL-6, IL-1 β and TNF- α .^{66, 21} Wang et al. could detect a marked increase in TNF- α expression 1 week after one session of ALA-PDT in an SCC mouse model.⁶¹ In ALA-PDT experiments performed on cultured keratinocytes, derived from photodamaged skin, significantly increased levels of IL-1 α , IL-6 and TNF- α could be detected in the keratinocyte supernatant 24 hours after PDT. These cytokines not only mediate antitumor immune response but decrease the expression of matrix metalloproteinases (MMP-1) and increase collagen synthesis. In this way, these cytokines have a role in photorejuvenation as well.⁶⁹ In healthy human skin, the expression of TNF- α was significantly increased 24 hours after PDT, but the production of IL-8 and IL-1 β was unchanged following PDT.⁶³ Regarding IL-6, its level in peripheral blood after MAL-PDT did not change significantly in AK patients.⁷⁰

It is still not known what cytokines are elevated or decreased in AK, nor how PDT might alter their concentrations. Regarding the immunosuppressive effect of PDT, another interesting question would be, how PDT change the concentration of IL-10, TGF- β and how this influences treatment outcomes and recurrence rates?

Is the adaptive immune system involved in the effect of PDT?

The number of CD4⁺ and CD8⁺ T cells were increased at 4 hours after PDT in healthy human skin.⁶³ At 24 hours⁷¹ and one week after ALA-PDT in an SCC mouse model, the amount of CD4⁺ and CD8⁺ T cells was significantly increased.⁶¹ Long-term tumor control after PDT is thought to be achieved by CD8⁺ anti-tumor T cell response, whereas CD4⁺ T cells play only a supportive role.⁷² Only one human study is available which intended to examine the number of Tregs in peripheral blood after MAL-PDT of AKs. The authors found that MAL-PDT did not influence significantly the number and even the function of Tregs in patients with multiple AK.⁷⁰

Other human studies analyzing the composition of T cells in AKs or the influence of PDT on T cells in human AK samples are not yet available. It would be important to evaluate these changes to gain real information about the mechanism of action of PDT on AKs, and to possibly explain the occasionally observed therapeutic failure. Moreover, in terms of healing, the dynamics of the immune response (immune activation, immunosuppression and anti-inflammatory events) after treatment is also very important, but remains unknown at the present time.

Conclusions and future outlook

In addition to UV-induced DNA damage, UV-induced immunosuppression and inflammation are also essential in the development of AK and cSCC. The immune cells are well characterized in photodamaged skin, IEC and cSCC and partially in AK. During progression of AK to cSCC, the composition of immune cells is changing, giving opportunity to find prognostic tools, like CD4⁺/CD8⁺ ratio, although more human studies would be needed to identify an immune marker which would predict therapeutic efficacy. The major events induced by PDT are extensively studied in non-skin cancer models both in humans and animals, while the immunological effects of 5-ALA/MAL PDT on AK, IEC and cSCC lesions have been mainly investigated in mouse models. Experimental data in human are missing. It is very likely that the success of photodynamic therapy depends on the immune cell's composition in actinic keratosis before treatment and that long-term benefit of photodynamic therapy depends on its immunological effects. To achieve a better lesion selection for photodynamic therapy and to develop appropriate therapeutic combinations, further studies evaluating the effects of PDT on neutrophils, DCs, cytokines and T cells in epithelial precancerous and cancerous lesions are needed in the future.

Conflict of interest

The authors have no conflict of interest.

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Figure 1

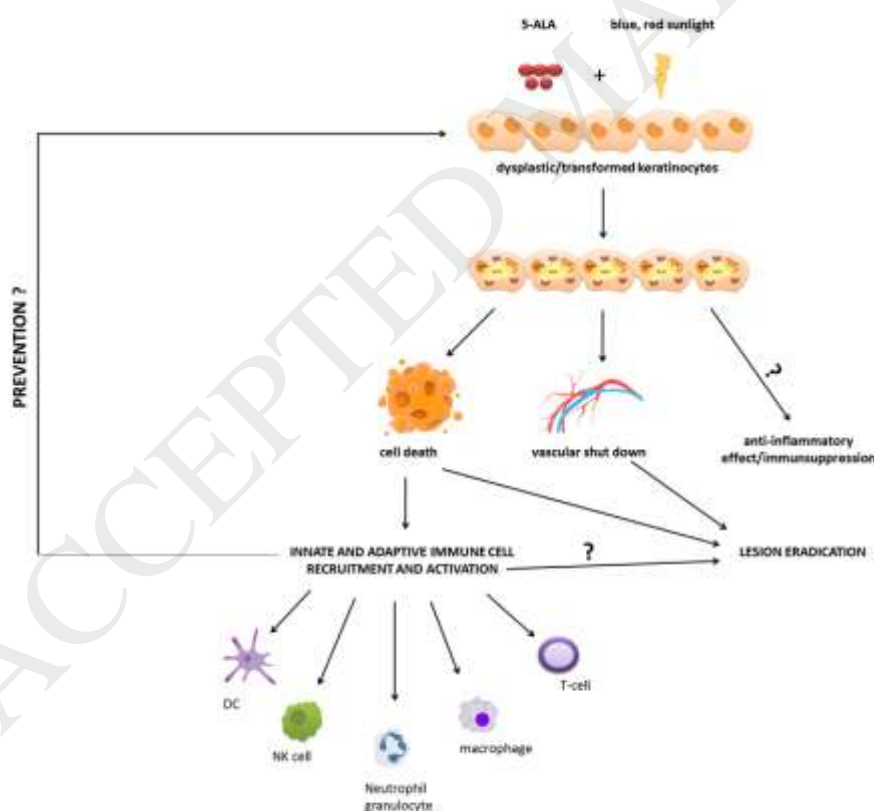


Table 1. Distribution of immune cells in human photodamaged skin, actinic keratosis, intraepidermal carcinoma and squamous cell carcinoma

		Photodamaged skin	Actinic keratosis	Intraepidermal carcinoma	Squamous cell carcinoma
<i>CD1a⁺ Langerhans cells</i>	Takahara et al.¹⁷	NI	highest expression	moderate expression	lowest expression
<i>Conventional dendritic cells</i>	Freeman et al.¹⁹	highest expression	NI	lowest expression	low expression
	Jang et al.¹⁸	NI	lowest expression	higher expression	highest expression
<i>Plasmacytoid dendritic cells</i>	Freeman et al.¹⁹	low expression	NI	low expression	high expression
<i>CD3⁺ T cells</i>	Hussein et al.¹⁶	NI	high expression	NI	moderate expression
	Freeman et al.¹⁹	lowest expression	NI	highest expression	moderate expression
<i>CD4⁺ T cells</i>	Freeman et al.¹⁹	moderate expression	NI	higher expression	highest expression
<i>CD8⁺ T cells</i>	Freeman et al.¹⁹	moderate expression	NI	moderate expression	low expression
<i>TIA-1⁺ cytotoxic T cells</i>	Hussein et al.¹⁶	NI	moderate expression	NI	high expression
<i>CD56⁺ NK cells</i>	Freeman et al.¹⁹	high expression	NI	low expression	low expression
<i>CD68⁺ macrophages</i>	Hussein et al.¹⁶	NI	moderate expression	NI	high expression
	Takahara et al.¹⁷	NI	lowest expression	moderate expression	highest expression
<i>CD20⁺ B cells</i>	Hussein et al.¹⁶	NI	moderate expression	NI	high expression
<i>Regulatory T cells</i>	Freeman et al.¹⁹	moderate expression	NI	moderate expression	moderate expression
	Jang et al.¹⁸	NI	lowest expression	higher expression	higher expression
<i>$\gamma\delta$ T cells</i>	Freeman et al.¹⁹	moderate expression	NI	moderate expression	moderate expression

NI: not investigated

Table 2. Influence of photodynamic therapy on immune cells

	<i>Langerhans cells</i>	<i>Conventional Dendritic cells</i>	<i>CD4+ T cells</i>	<i>CD8+ T cells</i>	<i>Neutrophils</i>	Model
Hayami et al. ³⁶	decreased 24 hours after PDT on the treated site	increased in the draining lymph nodes 24 hours after PDT				murine contact hypersensitivity model (CHS)
Wang et al. ⁶¹	increased 24 hours after ALA-PDT on the treated site		increased 1 week after PDT	increased 1 week after PDT		SCC mouse model
Evangelou et al. ⁶³	decreased 24 hours after PDT on the treated site		increased 4 hours after PDT	increased 4 hours after PDT	4 hours after PDT increased, but 24 hours later decreased	healthy human skin
Nakaseko et al. ⁶⁸					3 hours later increased, but 3 days after PDT decreased	human actinic keratosis
Bhatta et al. ⁷¹			increased 24 hours after PDT	increased 24 hours after PDT		SCC mouse model